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CONTENTS.

PAGE

Arthur, D. R.

Some Aspects of the Ecology of the Tick, *Ixodes ricinus*, L., in Wales (*illustrated*) 321

Beament, J. W. L.

The Penetration of the Insect Egg-shells. I.—Penetration of the Chorion of *Rhodnius prolixus* Stål (*illustrated*) 359

Beament, J. W. L.

The Penetration of Insect Egg-shells. II.—The Properties and Permeability of Sub-chorial Membranes during Development of *Rhodnius prolixus* Stål (*illustrated*) 467

Borkhsenius, N. S.

Notes on *Pseudococcus comstocki* (Kuw.) and some allied Species (Homoptera : Coccoidea), with Descriptions of three new Species 417

Callan, E. McC.

Effect of Defoliation on Reproduction of *Cordia macrostachya* ... 213

Cockings, K. L.

Successful Methods of trapping *Trombicula* (Acarina) with Notes on rearing *T. deliensis*, Walch (*illustrated*) 281

Dakshinamurty, S.

The common House-fly, *Musca domestica*, L., and its Behaviour to Temperature and Humidity (*illustrated*) 339

Evans, J. W.

A new Species of *Erythroneura* from Fiji (Homoptera, Jassidae) (*illustrated*) 131

Garnham, P. C. C.

Acrodendrophilic Mosquitos of the Langata Forest, Kenya... ... 489

Haddow, A. J.

The Mosquitoes of Bwamba County, Uganda. VI.—Mosquito breeding in Plant Axils (*illustrated*) 185

Hadjinikolau, J. & Busvine, J. R.

Toxicity of Limewash containing DDT or "Gammexane" to Mosquitos (*Aëdes aegypti*, L.) (*illustrated*) 179

Hafez, M.

A simple Method for breeding the House-fly, *Musca domestica*, L., in the Laboratory 385

CONTENTS.

PAGE

Hinton, H. E.
A Synopsis of the Genus *Tribolium* Macleay, with some Remarks on the Evolution of its Species-groups (Coleoptera, Tenebrionidae) (*illustrated*) 13

Hocking, K. S. & MacInnes, D. G.
Notes on the Bionomics of *Anopheles gambiae* and *A. funestus* in East Africa (*illustrated*) 453

Jackson, C. H. N.
Some further isolated Generations of Tsetse Flies 441

Jepson, W. F.
An annotated List of Insects associated with Ground-nuts in East Africa 231

Kapur, A. P.
On the Old World Species of the Genus *Stethorus* Weise (Coleoptera, Coccinellidae) (*illustrated*) 297

Kapur, A. P.
On the Indian Species of *Rodolia* Mulsant (Coleoptera—Coccinellidae) (*illustrated*) 531

Laws, S. G.
A biological Test for assessing the acaricidal Properties of DDT and "Gammexane" Preparations (*illustrated*) 277

Leeson, H. S. & Theodor, O.
Mosquitos of Socotra (*illustrated*) 221

Lewis, D. J.
The Mosquitos of the Jebel Auliya Reservoir on the White Nile (*illustrated*) 133

Lewis, D. J.
Glossina tachinoides in North-east Africa 529

Macan, T. T.
Mosquitos and Malaria in the Kabaw and Kale Valleys, Burma (*illustrated*) 237

Morris, K. R. S. & Morris, M. G.
The use of Traps against Tsetse in West Africa (*illustrated*) 491

Notley, F. B.
The *Leucoptera* Leaf Miners of Coffee on Kilimanjaro. I.—*Leucoptera coffeella*, Guér. (*illustrated*) 399

Parker, A. H.
Stimuli involved in the Attraction of *Aëdes aegypti*, L., to Man (*illustrated*) 387

CONTENTS.

PAGE

PARNELL, F. R., KING, H. E. & RUSTON, D. F.
Jassid Resistance and Hairiness of the Cotton Plant (*illustrated*) ... 539

RIPPER, W. E. & TUDOR, P.
The Development of a Helicopter Spraying Machine (*illustrated*) ... 1

ROBERTS, F. H. S. & O'SULLIVAN, P. J.
Studies on the Behaviour of Adult Australasian Anophelines (*illustrated*) 159

SIMMONDS, F. J.
The effective Control by Parasites of *Schematiza cordiae*, Barber, in Trinidad (*illustrated*) 217

SIMMONDS, F. J.
Some Difficulties in determining by Means of Field Samples the true Value of Parasitic Control 435

THEODOR, O.
Classification of the Old World Species of the Sub-family Phlebotominae (Diptera, Psychodidae) (*illustrated*) 85

WALOFF, N.
Development of *Ephestia elutella*, Hb. (Lep., Phycitidae) on some natural Foods (*illustrated*) 117

WILSON, S. G.
A Method of assessing the acaricidal Properties of DDT and "Gammexane" Preparations in Field Trials (*illustrated*)... ... 269

WILSON, S. G.
The feeding of "Gammexane" and DDT to Bovines (*illustrated*) ... 423

WRIGHT, D. W. & GEERING, Q. A.
The Biology and Control of the Pea Moth, *Laspeyresia nigricana*, Steph. (*illustrated*) 57

ERRATA.

Vol. **38**, 1947.

Page 437, 3 lines from end, for " feeds less frequently than the latter under moist conditions, but more " read " feeds more frequently than the latter under moist conditions but less "

Page 575, Table I.

Column 4 should read	Column 5 should read
500	50*
300	75*
—	—
—	—
—	—
4–7·5	3–7·5
0·4–1·2	—
0·6–0·7	—
	—

Page 576, Table II.

Column 3 should read	Column 4 should read
12–75	5–30
<50–<75	0·4–7·5
0·35–2	30 (approx.)
0·2–10	—

Vol. **39**, 1948.

Page 11, 4 lines from end, for " wind " read " wing "

Page 55, 7 lines from end, for " Bull. U.S. Dep." read " Tech. Bull. U.S. Dep."

Page 84, 6 lines from end, for " spg," read " sp." and line 5, for " Berücksichtigun " read " Berücksichtigung "

Page 85, line 5 of Contents, for " species " read " subgenera "

Page 143, for " *Culex* (*Culicomyia*) " read " *Culex* (*Culiciomyia*) "

Page 155, 4 lines from end, for " Trewevas " read " Trewavas "

Page 297, line 14, for " twenty " read " twenty-one "

Page 298, line 8, for " *S. malayensis* " read " *S. truncatus* "

Page 335, 9 lines from end, for " form " read " former "

Page 337, 8 lines from end, for " Vet. J." read " Vet. Rec."

Page 356, line 21, for " of low humidity " read " and low humidity "

Page 397, line 3, for " human surface body " read " human body surface "

Page 406, 22 lines from end, for " *Teleopterus leucopterae* " read " *Teleopterus violaceus* "

Page 437, the last equation should read

$$P=\frac{bp}{bp+a\ (100-p)}+100$$

Page 438, line 4 should read

$$\therefore\ 795p+63760=1500p$$

Page 465, line 1 of References, for " OLLINGER " read " OLINGER "

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BULLETIN

OF

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Vol. **39.** 1948.

THE DEVELOPMENT OF A HELICOPTER SPRAYING MACHINE.

By W. E. Ripper, Ph.D., F.R.E.S. and P. Tudor.

(Pls. I–VIII.)

Introduction.

Apart from the final harvesting of agricultural crops, most cultivation work is completed before the vegetation has reached its maximum height, and consequently ordinary farm implements are not designed to compass very high crops. For pest control work, however, it becomes necessary to enter crops at late stages because insect pests and plant diseases often infest them at the height of their growth.

This requirement led to the development of the high clearance tractor which enabled crops in full vegetation to be treated, without damage, at a stage when no other agricultural implements could enter. But even these machines, with clearances of four to seven feet, will cause damage to certain crops grown for seed such as beet, Brassica and peas, when they become intertwined or wind blown at the later stages of growth.

A logical development from the high clearance tractor was, therefore, the aeroplane. At first thought, it might be supposed that a country in a leading position in aviation, such as Great Britain, would also take the lead in aerial spraying. The advantages of aerial spraying are great, and include avoidance of damage to crop irrespective of growing conditions, high speed of operations, and independence of ground conditions.

Aeroplane dusting and spraying have not become the practice in Great Britain largely on account of the danger of drift of the dust or spray to adjacent fields. Even in America, where the fields are extensive, this danger cannot be avoided but in Great Britain it is greatly enhanced by the smallness of the fields. In addition, wet spraying of crops by aeroplane was not developed in U.S.A. to any serious degree until the last few years. Dusting for agricultural and orchard crops in England, even with ground equipment, is not as effective as spraying chiefly because a dust coverage is greatly affected by wind and rain. The use of dust has consequently been found uneconomic except in cases where it effects an immediate contact action. On the contrary, the development of spray chemicals in Britain has shown a trend towards the achievement of greater resistance to rain than is usual in spray chemicals used in other parts of the world.

There is another point. Even in America the accident rate on aeroplane dusting operations is very high and it is obvious that in the more densely populated country of Great Britain with its abundance of trees, telephone and telegraph wires bordering the fields, the flying of aeroplanes at 70 to 100 miles per hour at 4 to 6 feet above ground, would constitute a much greater danger.

In a study of the possibilities of aerial spraying, in which the American experience of spraying by aeroplane and autogiro was taken into consideration, it was decided, in 1942, that, for use in England, the helicopter offered a more promising vehicle for an aerial spraying machine. The helicopter, like an aeroplane, can be used to discharge dusts and liquids into the air and its slip-stream can be relied upon to deposit the spray droplets on to the foliage beneath. It has the advantage that it does not require a prepared landing ground, can be flown at a much lower speed and has greater manoeuvrability in a small air space. Trees and high tension wires do not constitute such dangerous obstacles. American operators used helicopters in late 1945 and 1946 for dusting and there is one report of rotary wing aircraft being used for spraying. It is a generally acknowledged fact, however, that aircraft dusting and spraying carried out in this way does not give results comparable in quality with those achieved by ground equipment.

Yuill, Deonier, Wisecup and Girdler (1946) studied the possibilities of using a helicopter for applying DDT sprays for the control of malaria-carrying mosquitos. The helicopter was flown at a height of 30 to 80 ft. while spraying and was used in much the same way as an aeroplane is accustomed to be used for such work. A great deal of interesting data was obtained; in particular, measurements of the effective swathe widths were carried out at a considerable forward speed to compare the efficiency of the helicopter with that of a fixed wing aircraft. No study, however, of the use of the interference between the spray laden slip-stream of the helicopter and the vegetation seems to have been attempted.

Theoretical considerations, experiments on models and full-scale experiments showed that the helicopter slip-stream at certain speeds, on impact with the ground, first attains a horizontal direction and later in part rebounds. It seemed probable, therefore, that a spray-laden slip-stream could be used to wet a good number of the lower surfaces of leaves during its horizontal and upward travel, provided that the aircraft flew sufficiently low to effect ground interference whilst the slip-stream velocity was still high.

It was decided that an attempt should be made to use the interference of the helicopter slip-stream with the vegetation to cause the precipitation, by impact, of a considerable quantity of spray droplets and to effect a high dynamic catch which would result in a spray deposit similar or superior to that achieved by ground equipment. At the same time it seemed likely that drift losses would be smaller than is the case in aeroplane spraying. The following is a preliminary report on investigations carried out in 1945 and 1946.

The authors wish to acknowledge the help received in 1945 from Mr. H. M. Garner, Director of Scientific Research, and Capt. R. N. Liptrot, of the Ministry of Aircraft Production, in lending an R.A.F. R.4 helicopter; from Squadron Leader Marsh and his staff at the rotary wing station at Henley in flying the helicopter and assisting with the first year's trials; and in 1946 the further help from Capt. Liptrot, then of the Ministry of Supply, who hired them an R.A.F. helicopter. Thanks are also due to Sir John Fryer and Dr. R. A. E. Galley of the Agricultural Research Council, who recommended the request for this charter. The authors are also indebted for advice and criticism to Mrs. M. Glauert, Mr. C. G. Pullin of Cierva Autogiro Company Limited, Mr. Squires of the Royal Aircraft Establishment, and Dr. R. A. E. Galley of the Agricultural Research Council.

Method.

An aerial spraying unit was set up and a series of systematic experiments laid out.

1. *Helicopter.*

The helicopter used for the purpose was a Sikorski type Y.R.4.B., the property of the R.A.F. While helicopters had been previously designed and flown in England, this was the first production model which became available for aerial work. It was designed as a trainer and its production was commenced before a number of " teething troubles " had been rectified so that maintenance was costly and complicated in this country through shortage of spares. Its important characteristics, according to the manufacturer's specifications, are :

(*a*) *Airofoil* (Rotor blades).

Blade area 21·83 sq. ft. giving total area for the three blades of 65·49 sq. ft. Rotor disc area 1,134 sq. ft. giving disc loading of 2·16 lb. per sq. ft. at a total weight of 2,450 lb. Fabric-covered blades each 19 ft. in length turning in a substantially horizontal plane to give both vertical and translational lift. Tail rotor : with three blades turning in vertical plane to counter the torque reaction and serve as rudder by varying pitch.

(*b*) *Engine.*

Warner Super Scarab type R-550/1 developing a maximum of 190 B.H.P.
Fuel consumption 10 to 12 gal. per hour.

(*c*) *Performance.*

Maximum power loading, 12·89 lb. per B.H.P. Rotor revolution 230 r.p.m. giving maximum velocity of air stream through rotor disc of 21·21 ft. per sec. Maximum vertical ascent : 100 ft. Speed : —15 to +75 m.p.h. with normal cruising speed of 55 to 60 m.p.h. Cruising range : 2-5 hours.

(*d*) *Weights.*

Empty weight : 2,136 lb. All up weight : 2,600 lb. If no passenger carried, useful load : 250 lb.

In the initial flights the helicopter was piloted by Squadron Leader Marsh and during the majority of the experiments by Flight Lieutenant Theilman. No accident of any kind was experienced. In all, 25 flying hours were completed on the work. The experiments were carried out at the R.A.F. Station, Henley, the R.A.F. Station, Bourn, the trial grounds of Pest Control Limited at Harston, the Soham Estate of Mr. A. H. Worth, Fleet, Spalding, and the Madingley orchards of Mr. N. B. Blow of Covent Garden.

2. *Spraying Apparatus.*

For the initial trial at Henley, a tractor-driven ground spraying machine, Bean Royal 20, was connected to the helicopter by hose-pipe 150 feet long and spraying gear was fitted to the helicopter. In addition, the slip-stream underneath the hovering helicopter was explored by discharging hand-lances and so marking its path by spray particles. While this method gave an indication of the slip-stream performance, it was obviously clumsy.

An experimental spraying apparatus for the helicopter was, therefore, designed which consisted of a high-pressure air-storage vessel, high-pressure gauge, pressure-reducing valve, liquid storage reservoir holding six gallons of spray liquid and a low-pressure air-cushion (4 gallons volume) with a low-pressure gauge, pilot-control valve, pipe-line to spray-bar, spray-bar equipped with conventional eddy-chamber nozzles (see fig. 1). This spraying apparatus was mounted inside the cockpit in place of and above the passenger's seat (Pls. I and II) with the spray-bar on a

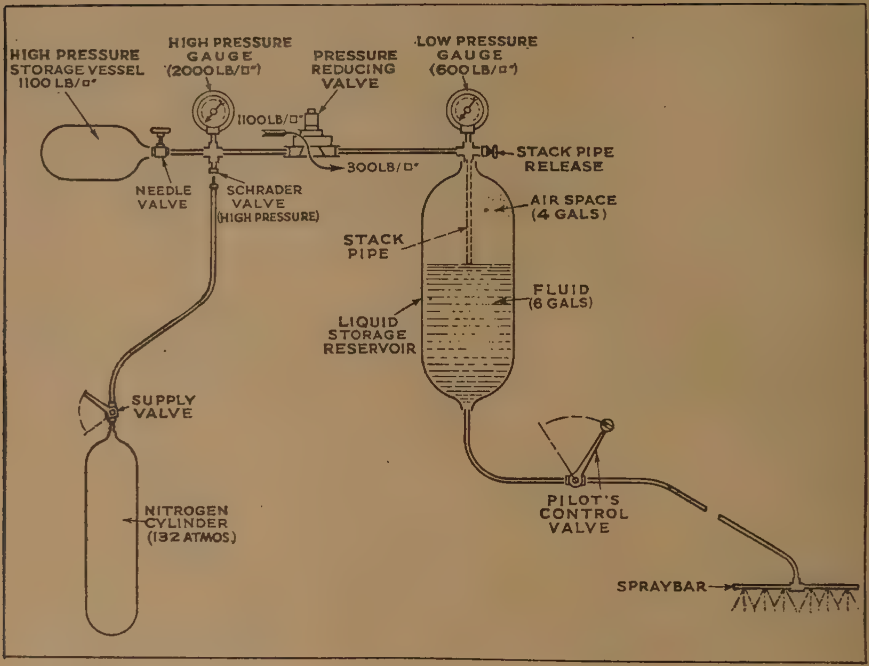

HIGH PRESSURE STORAGE VESSEL 1100 LB/□"
HIGH PRESSURE GAUGE (2000 LB/□")
PRESSURE REDUCING VALVE
LOW PRESSURE GAUGE (600 LB/□")
1100 LB/□"
STACK PIPE RELEASE
300 LB/□"
NEEDLE VALVE
SCHRADER VALVE (HIGH PRESSURE)
AIR SPACE (4 GALS)
STACK PIPE
FLUID (6 GALS)
LIQUID STORAGE RESERVOIR
SUPPLY VALVE
NITROGEN CYLINDER (132 ATMOS)
PILOT'S CONTROL VALVE
SPRAYBAR

Fig. 1.—Diagrammatic layout of experimental spraying gear as fitted to Y.R.4.B. helicopter.

cantilever type of support which allowed the use of three positions, in each of three different sectors of the annulus of the slip-stream (fig. 2). These positions were classified as follows :

" *A* " *position*—the spray-bar is placed in the most forward arc of the slip-stream annulus extending 15° either side of the longitudinal axis of the aircraft (fig. 3*a* and Pls. III & IV).

" *B* " *position*—in an arc of the slip-stream (fig. 3*b* and Pl. V) annulus 30° from the " A " position in an anti-clockwise direction so that the spray is discharged in the slip-stream over the arc 15° to 45° from the longitudinal axis of the aircraft, measured in an anti-clockwise direction (see fig. 3*b*).

" *C* " *position*—in an arc of the slip-stream annulus extending 15° either side of the perpendicular to the longitudinal axis of the aircraft (see fig. 3*c* and Pl. VI).

In all cases the spray-bar was adjustable in the horizontal plane so that discharge would take place at varying distances from the centre of the rotor hub, within the following limits :

In " A " position—between 10 ft. and 17 ft., the maximum lengths being 1A followed by 2A etc. to 5A the minimum length.

In " B " position—between 10 ft. and 18 ft., maximum length being termed 1B down to the minimum length 5B.

In " C " position—between 8 ft. and 16 ft., with a similar terminology 1C to 5C as above.

The position of the spray-bar could also be adjusted in a vertical direction to vary the distance of spray bar and the rotor disc ; the most remote from the rotor blades

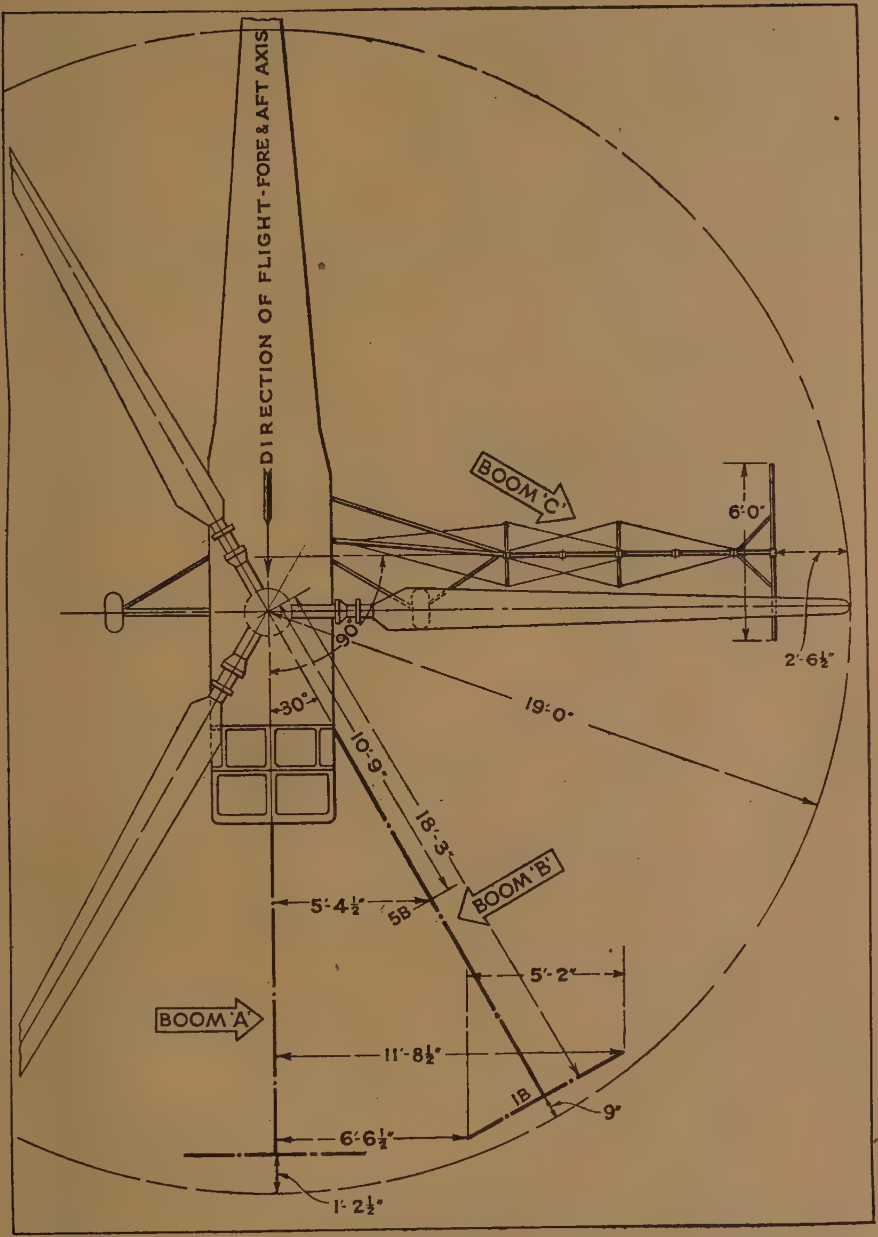
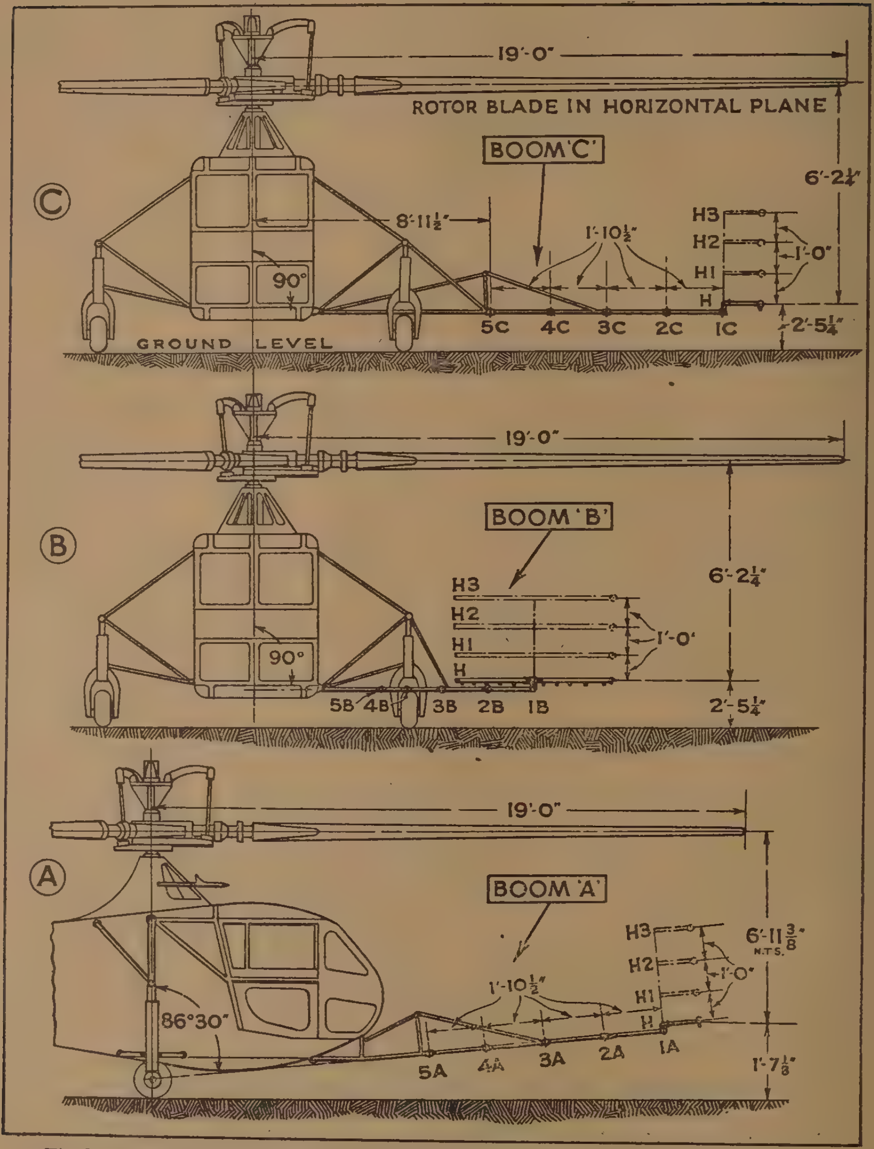

DIRECTION OF FLIGHT-FORE & AFT AXIS
BOOM 'C'
6'-0"
2'-6½"
19'-0"
90°
30°
10'-9"
18'-3"
BOOM 'B'
5'-4½"
5B
5'-2"
BOOM 'A'
11'-8½"
IB
9"
6'-6½"
1'-2½"

Fig. 2.—Plan showing three positions of spray boom in the annulus of the helicopter slip-stream.

was H1 followed by H2, H3 and, closest to rotor blades, H4, within the following limits:

A position—7 ft. 5 ins. to 4 ft.
B position—6 ft. 2 ins. to 3 ft. 2 ins.
C position—6 ft. 3 ins. to 3 ft. 2 ins.



C
19'-0"
ROTOR BLADE IN HORIZONTAL PLANE
BOOM 'C'
6'-2¼"
8'-11½"
1'-10½"
H3
H2
H1
H
1'-0"
90°
5C
4C
3C
2C
1C
2'-5¼"
GROUND LEVEL
B
19'-0"
BOOM 'B'
6'-2¼"
H3
H2
H1
H
1'-0"
90°
5B
4B
3B
2B
1B
2'-5¼"
A
19'-0"
BOOM 'A'
H3
H2
H1
H
1'-0"
6'-11⅜"
N.T.S.
1'-10½"
86°30"
5A
4A
3A
2A
1A
1'-7⅛"

Fig. 3.—Plan showing position of boom at different elevation in three positions A, B and C.

In the first place 2 per cent. summer oil emulsions were used as spray liquid to avoid evaporation of the spray droplets before they could reach the sensitive discs but it was found that, for slow speed helicopter spraying close to the ground, water was sufficient.

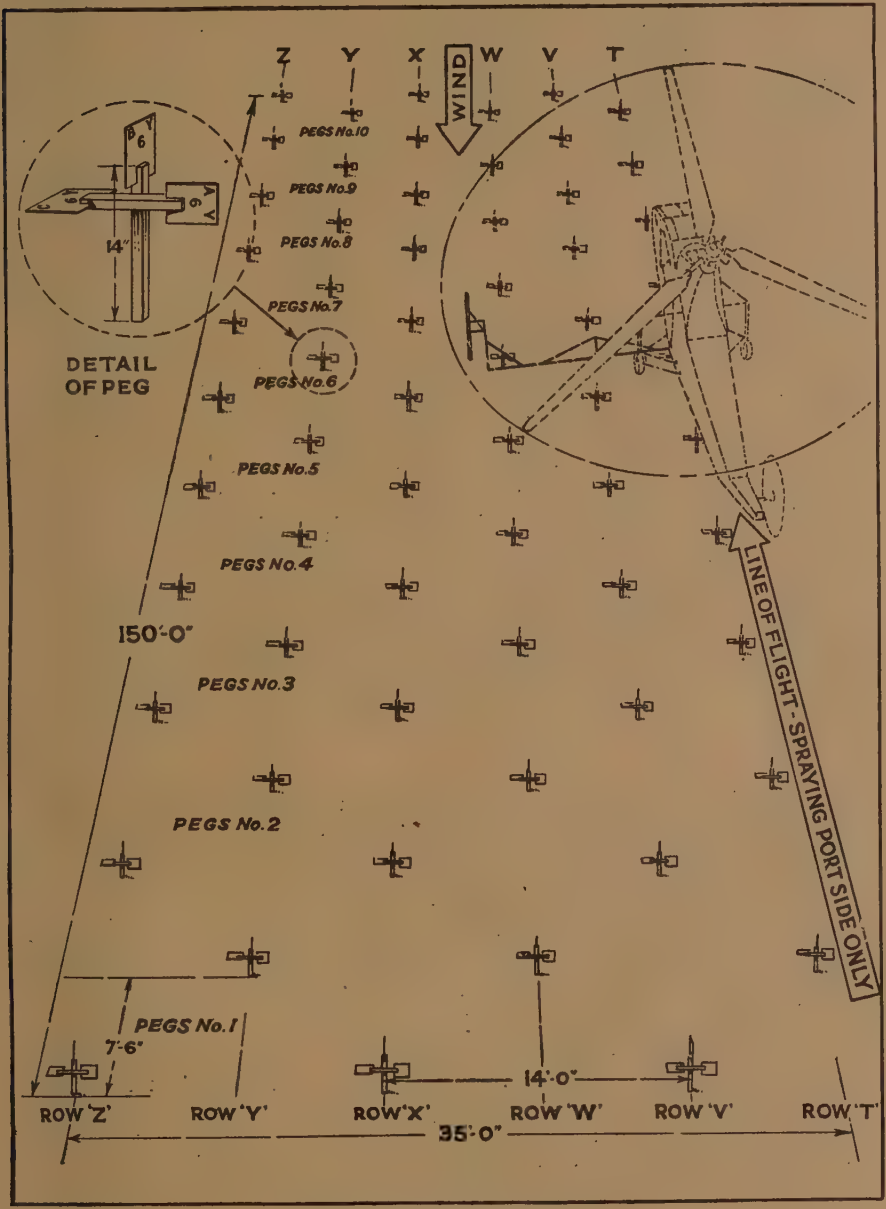

Z
Y
X
W
V
T
WIND
PEGS No.10
PEGS No.9
PEGS No.8
PEGS No.7
PEGS No.6
PEGS No.5
PEGS No.4
PEGS No.3
PEGS No.2
PEGS No.1
DETAIL OF PEG
14"
150'-0"
7'-6"
14'-0"
35'-0"
ROW 'Z'
ROW 'Y'
ROW 'X'
ROW 'W'
ROW 'V'
ROW 'T'
LINE OF FLIGHT - SPRAYING PORT SIDE ONLY

Fig. 4.—Layout of test plot.

3. *Recording Equipment.*

The apparatus used for recording results comprised a Bollex Paillard 16 mm. camera, a Casella portable 3 cup anemometer and sensitive indicator cards.

The sensitive indicator cards are a modification of a development by Cornelius Davies. They consist of pieces of 1/32 ins. white cardboard, measuring 2 ins. × 3 ins., treated with a solution of Kiton Green in carbon tetrachlorate, by immersing them in a solution for a few seconds and drying. The cards remain white but any water droplet deposited on the card changes the colour of the wetted area to malachite green.

All cards were numbered and lettered to signify the positions in which they were to be exposed on the field and relative to the perpendicular axis. The cards were exposed originally on wooden pegs but these were later replaced by metal holders (see fig. 4).

A test plot was set out by placing the pegs in a standardised pattern on the turf of the airfield or grassland on which treatment was to be given. The pattern of the layout and method of marking are seen on figure 4. In order to provide for change of wind, two plots were generally laid out, at right angles to each other.

Throughout the experiment the pegs were allowed to remain in the ground, fresh cards being inserted immediately before flight took place. The cards were collected immediately after the flight of the helicopter and graded according to a standard scale devised *ad hoc*, ranging from Grade 1 to Grade 6, Grade 6 containing cards showing complete cover.

After 4 gallons of spray liquid had been pumped into a liquid storage vessel, a commercial nitrogen cylinder was used to provide a pressure cushion in the liquid storage reservoir and in the high pressure vessel simultaneously by connecting it as shown in figure 1 ; the reducing valve allowed the liquid container to be pressurised to 300 lb. per square inch, while the high pressure cylinder was charged at 1,100 lb. per square inch. The aircraft then took off, attained constant height, and approached in a straight line the experimental plot, while the pilot tried to minimise his control movements. The helicopter flew over the field discharging its spray while the flight was timed by a stopwatch and the slip-stream filmed. The cards were removed as soon as they were dry, and replaced by clean ones ; the cards were then graded and analysed.

4. *Results.*

1). *Slip-stream of a helicopter.*

As it was intended that the slip-stream should be used as the medium for carrying the spray droplets to the position of final impact with the foliage, it was important to establish the performance of the main rotor slip-stream of the helicopter. Apart from the present investigation no research seems to have been done on the slip-stream path of a helicopter flying slowly in the ground cushion.

The experimental work was conducted by using liquids and smoke as markers, and by observing ripples when flying over stationary water surfaces. The slip-stream of the helicopter in its hovering position is bell-shaped, the cross-section at the height of the fusilage forming an annulus in which the difference between the outer and inner radii is approximately two-thirds of the rotor diameter. The centre is calm with a tendency to slight upstream and eddies.

When the helicopter flies slowly forward the cross-section of the annulus becomes elongated into a parabola-like curve with its apex forward in the direction of flight.

On impact with the ground, the slip-stream flattens out and spreads along the ground causing considerable turbulence rebounding.

In forward flying each rotor blade imparts to the slip-stream aft of the beam a vortex-like motion which results in layers of different speeds in the slip-stream behind the helicopter, and the turbulent air rises as the helicopter recedes; consequently any spray introduced into that part of the slip-stream would lead to stripy deposit. This phenomenon can be easily observed when the slip-stream is marked by smoke or when the helicopter is flying at a very low altitude over water.

2). *Performance of spray laden slip-stream.*

Experiments with various spray-bar positions showed that a good rebound can be obtained at distances of the spray-bar of between 5 and 6 ft. above the ground (Pl. VII). At lower heights, the slip-stream precipitates the spray, as it develops in cone shapes from the nozzles, straight into the ground with little rebound. From heights above 6 ft. a large proportion of the spray is driven by the slip-stream towards the ground and then drifts or trails away, without any interference with crops or ground, to settle finally under gravity (Pl. VIII).

The characteristics of the slip-stream are greatly affected by alteration of the pitch or azimuth control. For a uniform spray deposit it is, therefore, desirable to fly with as little alteration of the blade pitch as possible. In wind speeds up to 12 m.p.h. a useful spray deposit can be obtained provided the wind is not gusty, but cross winds make a certain amount of jarring of the helicopter unavoidable, and necessitate considerable pitch alteration if the cover is not to suffer. Accurate straight flying and accurate spray cover is, therefore, more difficult. Apart from effect caused by control movements, steady cross winds do not appreciably affect the spray up to 45° from the direction of flight.

The forward speed of the aircraft should not exceed 12 m.p.h. to ensure maximum re-bounding of the spray-laden slip-stream. The spray is deposited by the rotor slip-stream over a strip extending 35 to 40 ft. either side of the aircraft so that a total swathe of 70 ft. can be covered.

3). *Comparison of different parts of slip-streams for spraying purposes.*

It was one of the purposes of the investigation to obtain an indication of the most advantageous position of the spray-bars in relation to the aircraft with a view to evolving spraying equipment which would make full use of the interference of the slip-stream with the crop and the rebound of the dynamic catch of spray droplets on as large a proportion of the lower leaves as possible, because, for certain spray operations (application of fungicides and contact insecticides), a good cover of the lower leaf is indispensable.

Boom "A."

An analysis of the results obtained with the spray-bars in three different sectors of the slip-stream showed that in position A, the useful spread of spray liquid is not more than 14 ft. on each side of the longitudinal axis of the aircraft. It also showed that the most pronounced rebound is obtained when the spray-bar fitting is extended to its greatest length, that is, to a length of 18 ft. from the axis centre line of the rotor hub. The greatest rebound was obtained at a maximum speed of 7 m.p.h. Good results were obtained with relative wind speeds of 10 m.p.h. but above 10 m.p.h. intensity fell off rapidly. At speeds exceeding 10 m.p.h. the forward throw diminishes rapidly, the cover falls off and the spray becomes turbulent and much is thrown on the aircraft. Best results were obtained when the spray-bar was 5 to 6 ft. from the ground—if higher, the spray trails and the forward and outward throw suffers.

Steady cross winds do not seem to have a pronounced effect on this position so long as the relative wind speed does not exceed 12 m.p.h. and its direction is not more than 45° to either side of the course.

It is worth noting that in the H3 adjustment of the A1 position, the spray is projected 10 to 15 ft. forward of the rotor blade tip into a vortex which whirls the spray round at a height of 5 to 6 ft. above the ground. This fact might be utilised in the treatment of shrubs and small trees.

Boom "B."

This position gives the widest spread and the highest intensity values of cover at 1B. The maximum width of the swathe in this position is 33 ft. on either side. The average intensity value is 2·8 at 1B and 2·57 at 3B. For practical purposes this might be sufficient. Cross winds have no effect on the cover, so long as they are steady and their velocity does not exceed 12 m.p.h. Winds of higher velocity throw the spray back on the aircraft and prevent the swathe developing normally.

A large number of experiments show that the B position of the boom gives the most stable results of cover and is the least affected of the three positions by aircraft and control surface movements. The spray-bar in position 3B can be so moved that it will take on the work done by booms A and C. It would extend over an arc of 65° and be 16 ft. in length.

Boom "C."

Here again the first position 1C gives the best results with maximum intensity of 2·46 which is but slightly superior to 1A. All C boom positions produce spray-laden slip-streams with a preference for rebounding—a fact which is probably due to a slight trailing action. The formations of the spray in all C boom positions are sensitive to change in aircraft attitude and for this reason the deposit is more patchy. Cross winds affect the deposits in the same way as in other boom positions, except that with offplot winds the reversion becomes greater. Spray in fairly heavy quantities is deposited on the aircraft in position 1C.

If the spray-bar is brought nearer the rotor the spread increases but the deposit becomes patchy and inconsistent. Position 1C is considered impracticable in view of the marked effect on the deposit caused by small changes in aircraft attitude. A specification for an aircraft to be specially designed for helicopter spraying should provide for the minimum use of the azimuth control because a very small pitch or rolling movement will produce irregular spray formation and, not only change in the aircraft attitude, but momentary change in the control surface movement is sufficient to upset the spray deposit.

Discussion.

The experiments reported suffer from the disadvantage that they were obtained by the use of a low load-carrying helicopter which did not permit sufficient spray liquid to be carried to feed three spray-booms. It is, however, possible to superimpose the results of the three reported positions and thus forecast the spray pattern which will result from the use of a helicopter with a heavier load-carrying capacity spraying through a greater part of the slip-stream annulus. From a study of the results reported in this paper, and those obtained by superimposing the three sets, it is clear that the attempt to obtain spraying results comparable in quality with those obtained from up-to-date ground-spraying machines is handicapped by disappointing limitations which are inherent in the helicopter spraying machine: for good results the forward speed must be reduced as the spray deposits are affected by relatively low wind velocities when ground machines, equipped with wind-shields, can still operate.

To what extent these disadvantages are compensated by other obvious advantages, namely, the helicopter's greater speed from its base to the site of operations, independence of ground conditions, and ability to go through intertwined crops which cannot be entered without damage by ground equipment, remains to be seen.

There are other spray operations in which the rebound of the slip-stream is less important and where the helicopter can be flown at greater speed, but these are outside the scope of the present investigation. It is hoped that they will be dealt with in experiments with the Sikorski S.51 which is now being operated and which has been equipped with experimental and operational spraying gear based on the experience of the present report.

From rough calculations it seems doubtful whether a helicopter of the specification of the Bell 47D or the Sikorski S.51 would be an economic proposition for liquid spraying of certain crops. Nevertheless, experiments have convinced us that, under British conditions, spraying is preferable to dusting because (*a*) much less drift is experienced in spraying than in dusting and (*b*) in wet and windy weather, rainfast spray deposits bring the farmer much greater return for his investment in spraying.

In 1945 a specification was issued to a manufacturer of rotary wing aircraft for a helicopter with a pay load of 3 tons and an operational capacity which would allow travel over Great Britain, Europe and the African territories. It was stipulated that greater stability should be procured by the use of a multi-rotor system in order to minimise the movements of control surfaces which upset the deposit of spray liquid by the slip-stream. Pest control equipment for this heavy load-carrying helicopter is being designed for further experiments with this interesting aircraft.

Summary.

Experiments were carried out to study the possibilities of using ground or crop interference with the spray laden slip-stream of a helicopter flying slowly in the ground cushion to obtain a high dynamic catch of spray droplets. Experiments were carried out in 1945 and 1946 with a Sikorski type Y.R.4.B. helicopter and experimental spraying gear designed for the purpose.

The forward portion of the slip-stream annulus was explored by means of spray-bars placed in three different positions. The position in the sector between angles of 15° and 45° with the direction of forward flying was found to give the best all-round cover and was the position in which the spray deposit was less influenced by alterations in the control-surfaces of the helicopter.

To obtain a fairly wide swathe of approximately 70 ft., the equivalent of two rotor diameters, the forward speed of 6–8 m.p.h. was found to be optimal, and it was observed that not only was good cover of the upper surfaces of the leaves obtained but the spray-laden slip-stream also gave a good cover of the vertical surfaces of the plants outside the rotary periphery and, in certain boom positions, descended and in rebounding produced a cover of the lower surfaces.

In order to obtain maximum dynamic catch and a good cover of the lower surfaces of leaves, spray-bars must not be more than 6 ft. above ground.

Helicopter spraying is wind dependent and in wind velocities above 12 m.p.h. uniform cover of spray deposit does not appear to be feasible.

The performance of the flattened out slip-stream and rebound depends on the type of crop, and as this affects the dynamic catch to a considerable degree, this phenomenon will be the subject of a special investigation.

The forward portion of the slip-stream was found to give a more even spray deposit than the aft portion because in the latter case vortices are caused by the rotor blades which affect the spray deposit.

In view of its limitations as regards speed and dependence on wind velocity, economic application of spray chemicals by helicopter at a degree of efficiency and workmanship equal to that of ground machines is only possible with a heavy load-carrying helicopter. A specification for such a machine has been issued to the manufacturers of rotory wind aircraft.

It is hoped that this report will be followed by a report on the performance data of the new spraying apparatus designed for the Sikorski S.51 which is based on the results of these investigations.

References.

BROOK, A. (1947). The drifting of poisonous dusts applied by aeroplanes and hand rigs.—Agric. Engineering, **28** (B) pp. 233–239.

DAVIES, C. (1934). A method to determine the surface area of trees covered by spray fluid and to obtain a permanent record of the degree of fineness of the deposit.—J. S. E. agric. Coll., **34,** pp. 252–253.

KELLEY, F. H. (1947). Chemical application by helicopter.—Agric. Chemicals, **2,** pt. 7, pp. 36–39.

POTTS, S. F. (1939). Spraying woodlands with an autogyro for control of Gipsy Moth.—J. econ. Ent., **32,** pp. 381–387.

RIPPER, W. E. (1944). Improved means of aerial spraying B.P. 564771.

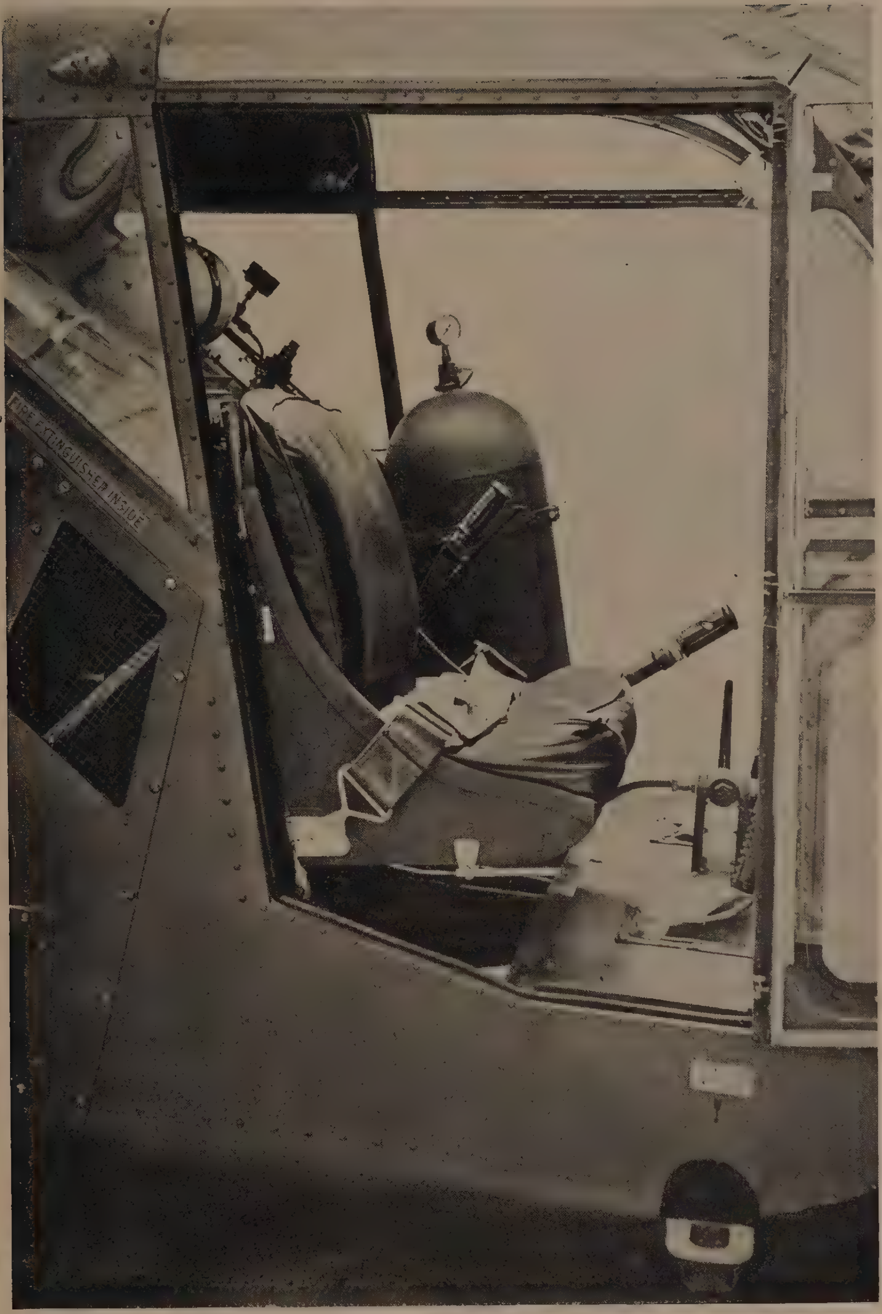
SEBORA, L. H., DEONIER, C. C., WISECUP, C. B., HUSMAN, C. N. & LONGCOY, O. M. (1946). Performance of aerial spray equipment used to disperse DDT at Orlando, Florida.—Mosq. News, **6,** pp. 169–177.

WHITTEN, R. R., POTTS, S. F. & FRANCIS, E. H. (1941). Concentrated spray applied with an autogiro for control of cankerworms.—J. econ. Ent., **34,** pp. 692–696.

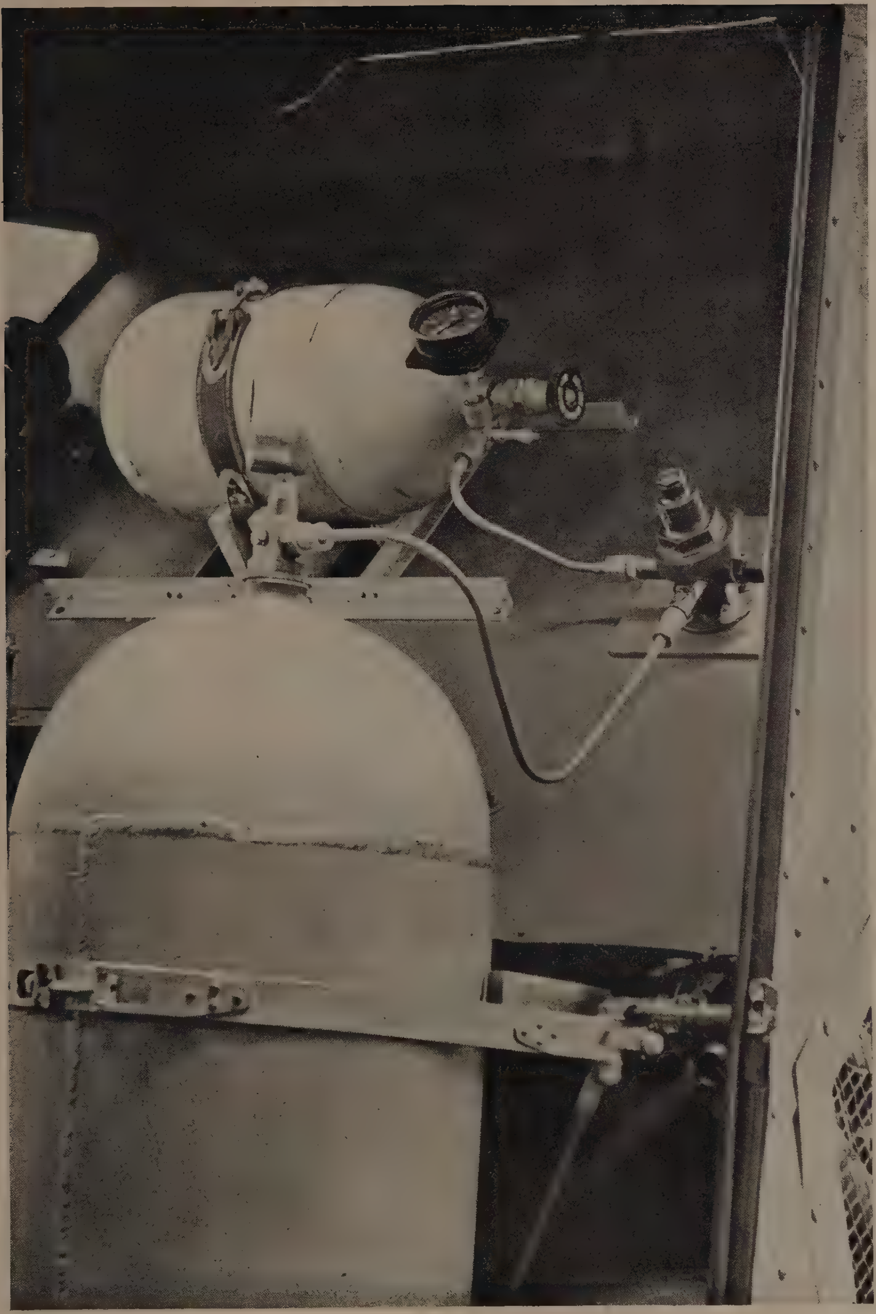
YUILL, J. S., DEONIER, C. C., WISECUP, C. B. & GIRDLER, G. W. (1946). Development of new spraying apparatus for HSN-1 Helicopter.—J. econ. Ent., **39,** pp. 729–734.

——, MADDEN, A. H., LINQUIST, A. W. & MACKEY, J. R. (1946). DDT sprays applied from a helicopter against adult mosquitos.—Proc. 14th Conf. Calif. Mosq. Contr. Ass., pp. 44–52, 93.

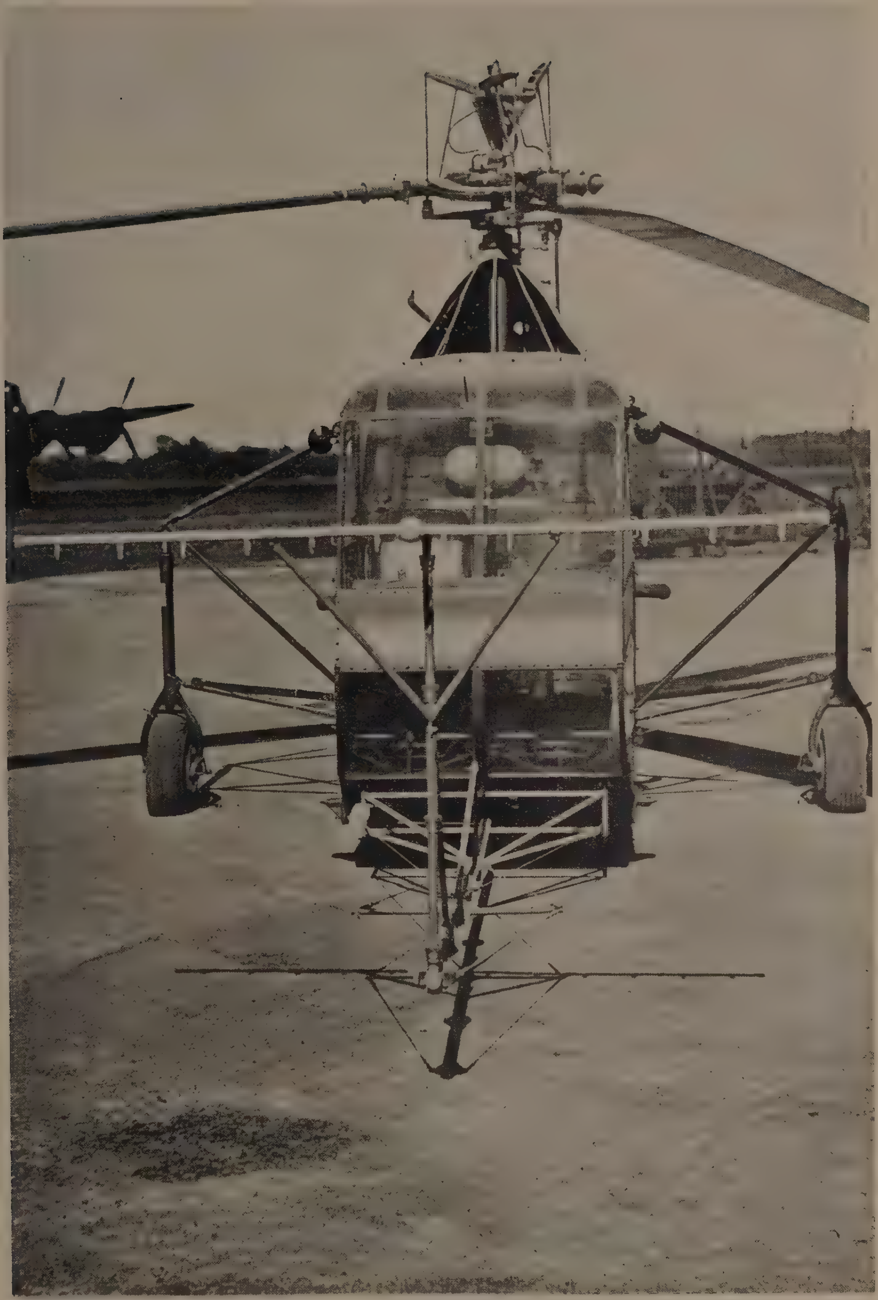
——, PARKER, D. E. & GERSHOITZ, D. (1946). Special report on application of spray containing DDT by helicopter.


FIRE EXTINGUISHER INSIDE

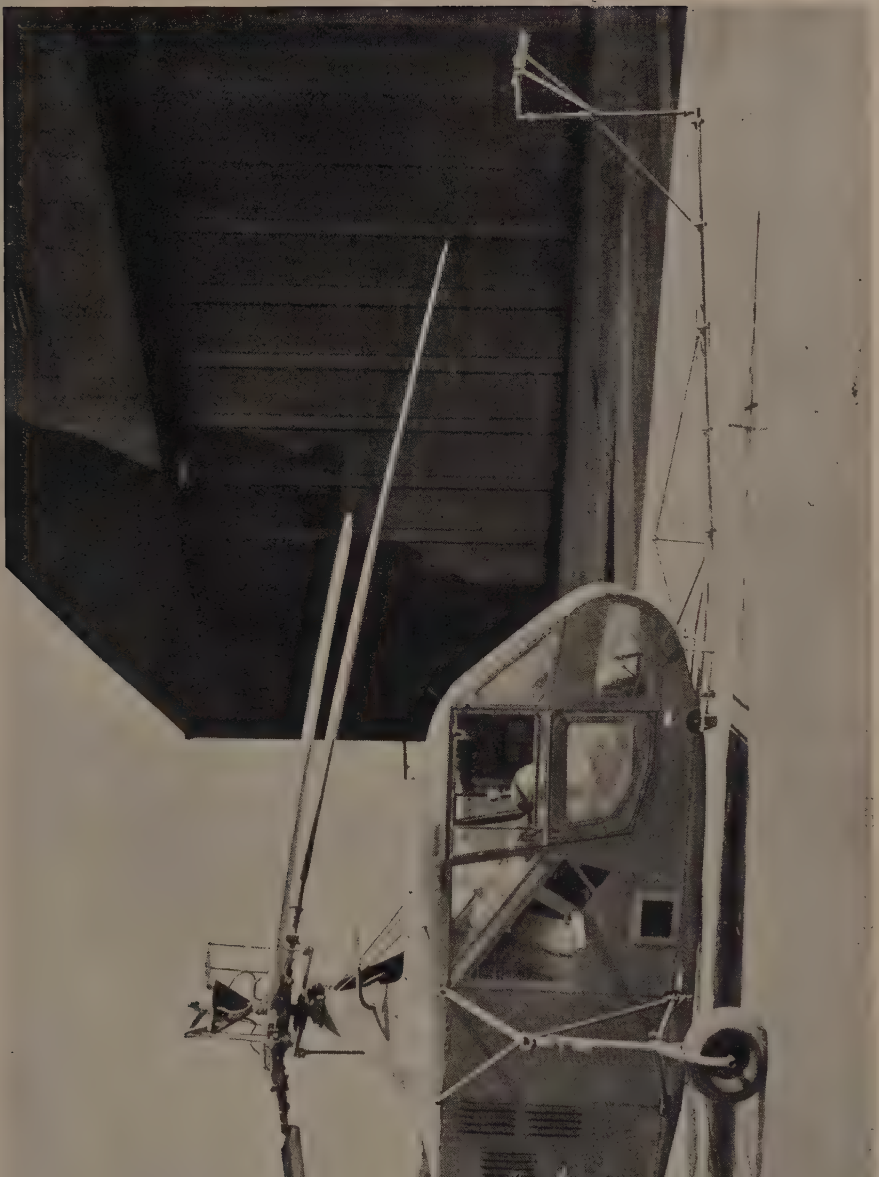
Cockpit of YR.4.B. helicopter showing spraying gear replacing passenger's seat.



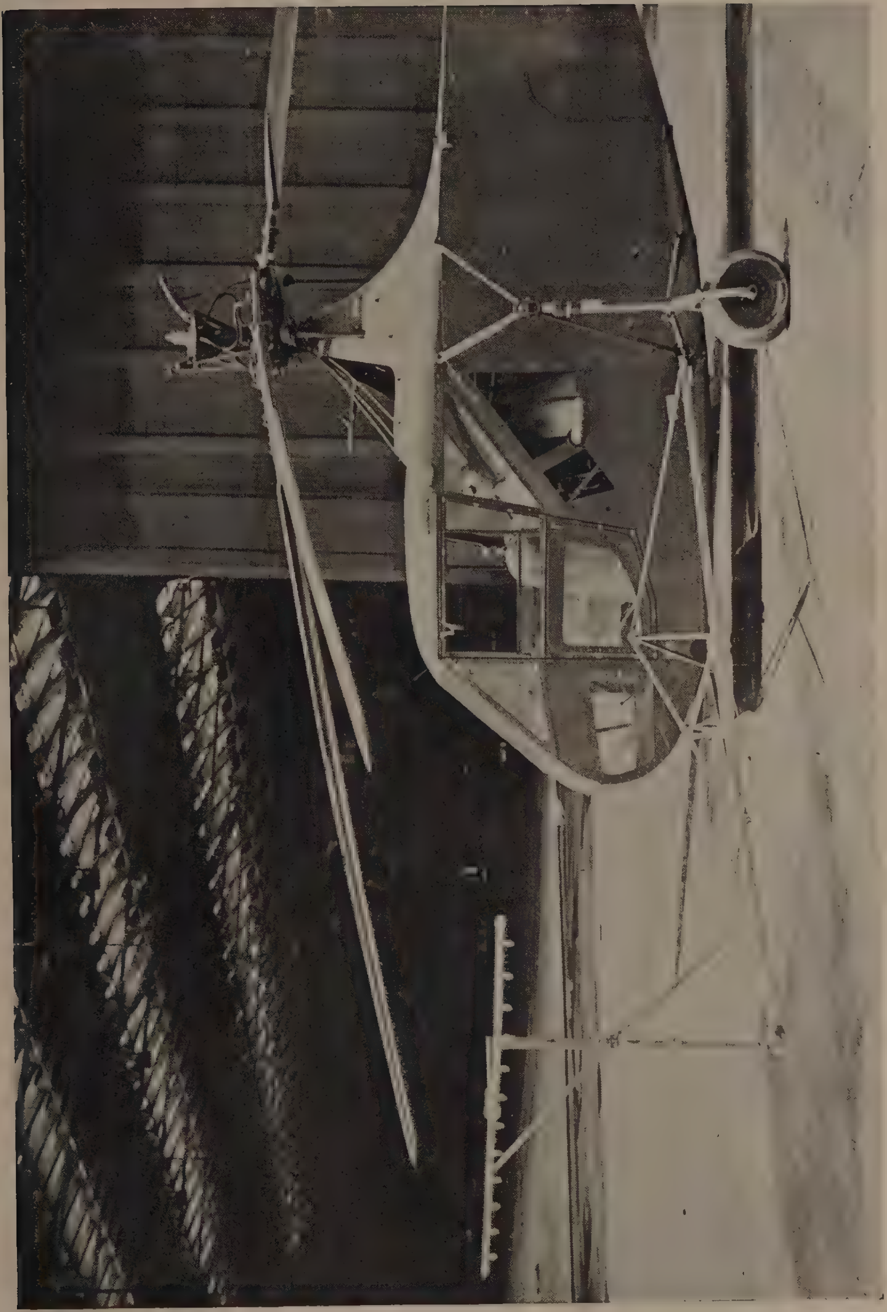
High pressure air storage vessel and liquid reservoir fitted in YR.4.B. helicopter.



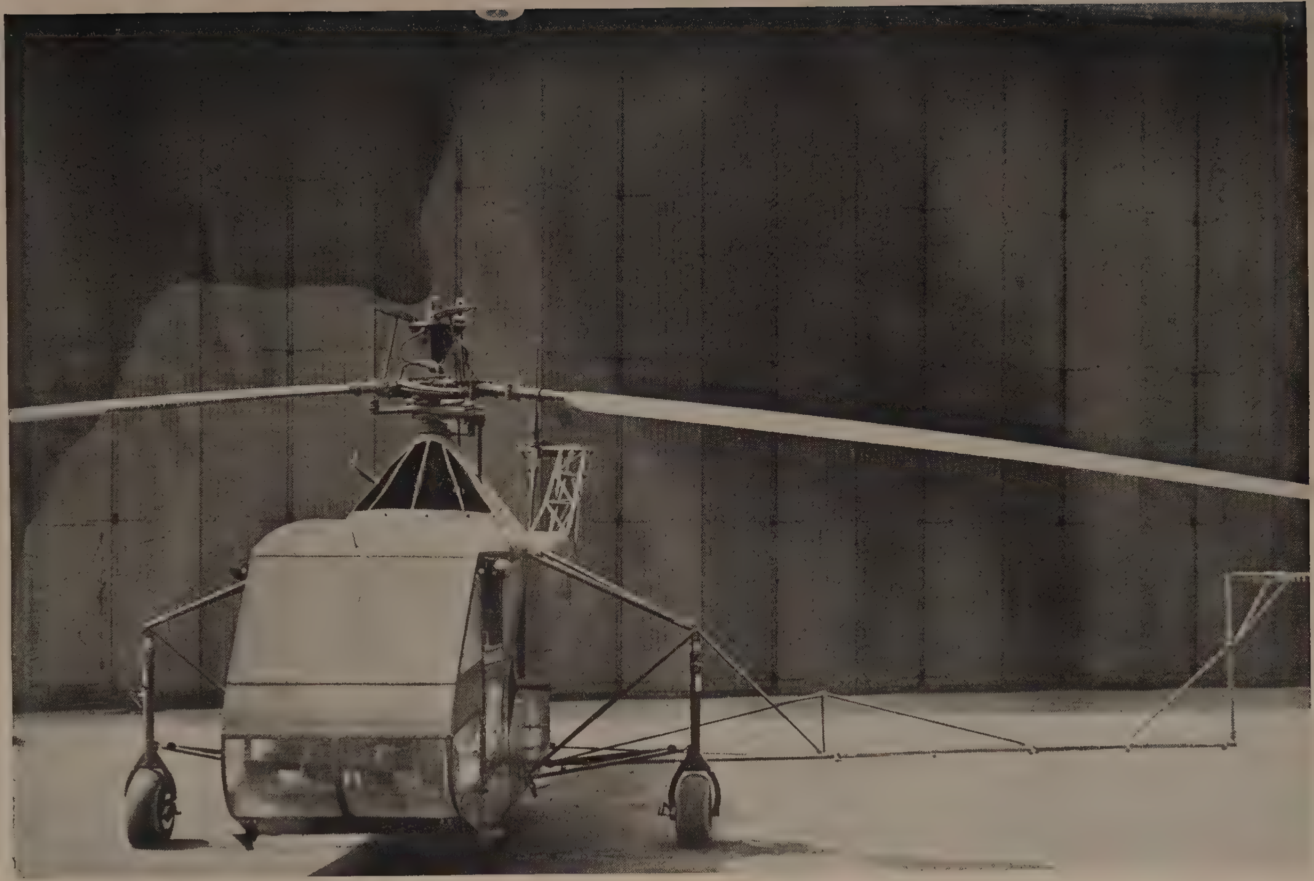
YR.4.B. helicopter with boom in " A " position.



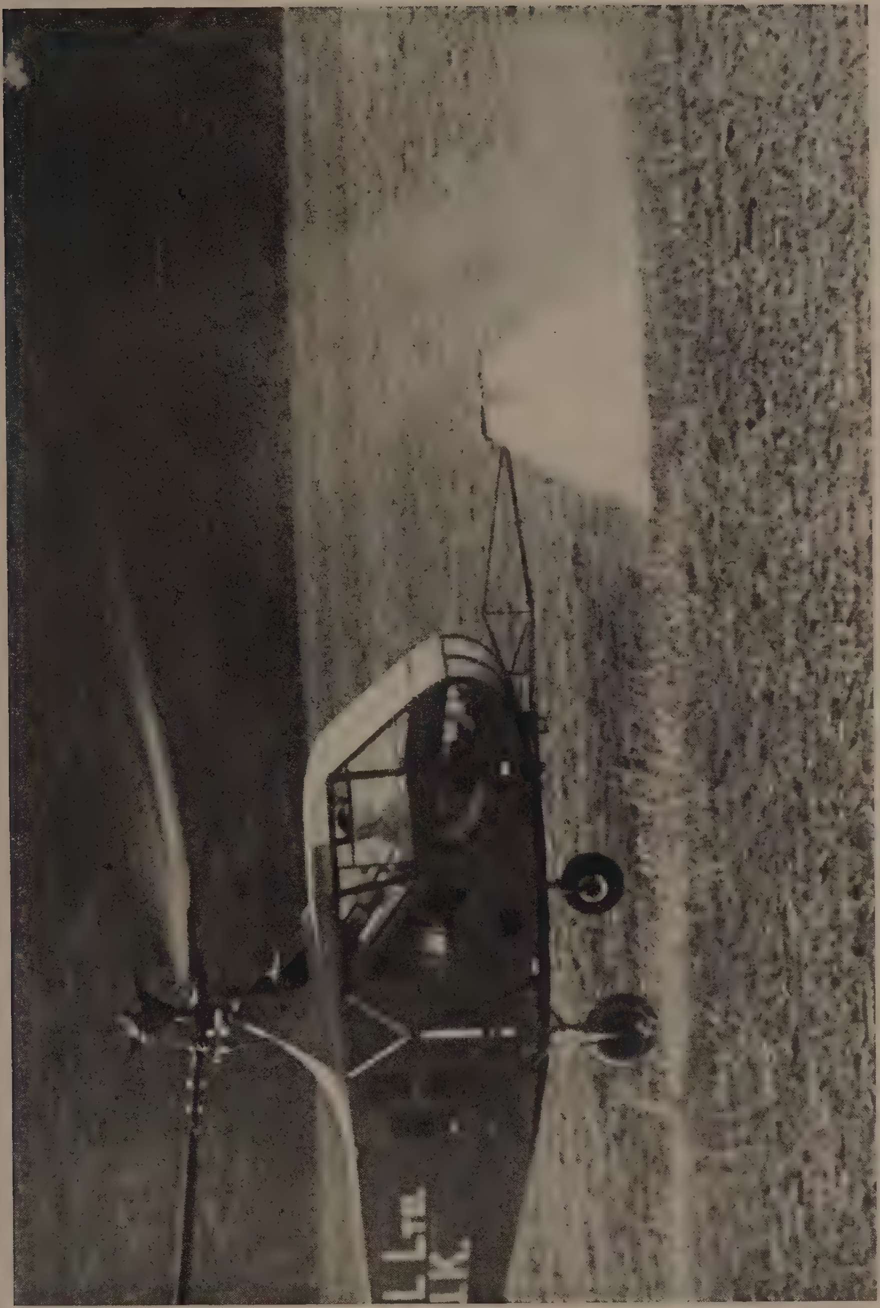
Side view of YR.4.B. helicopter with boom in "A" position.



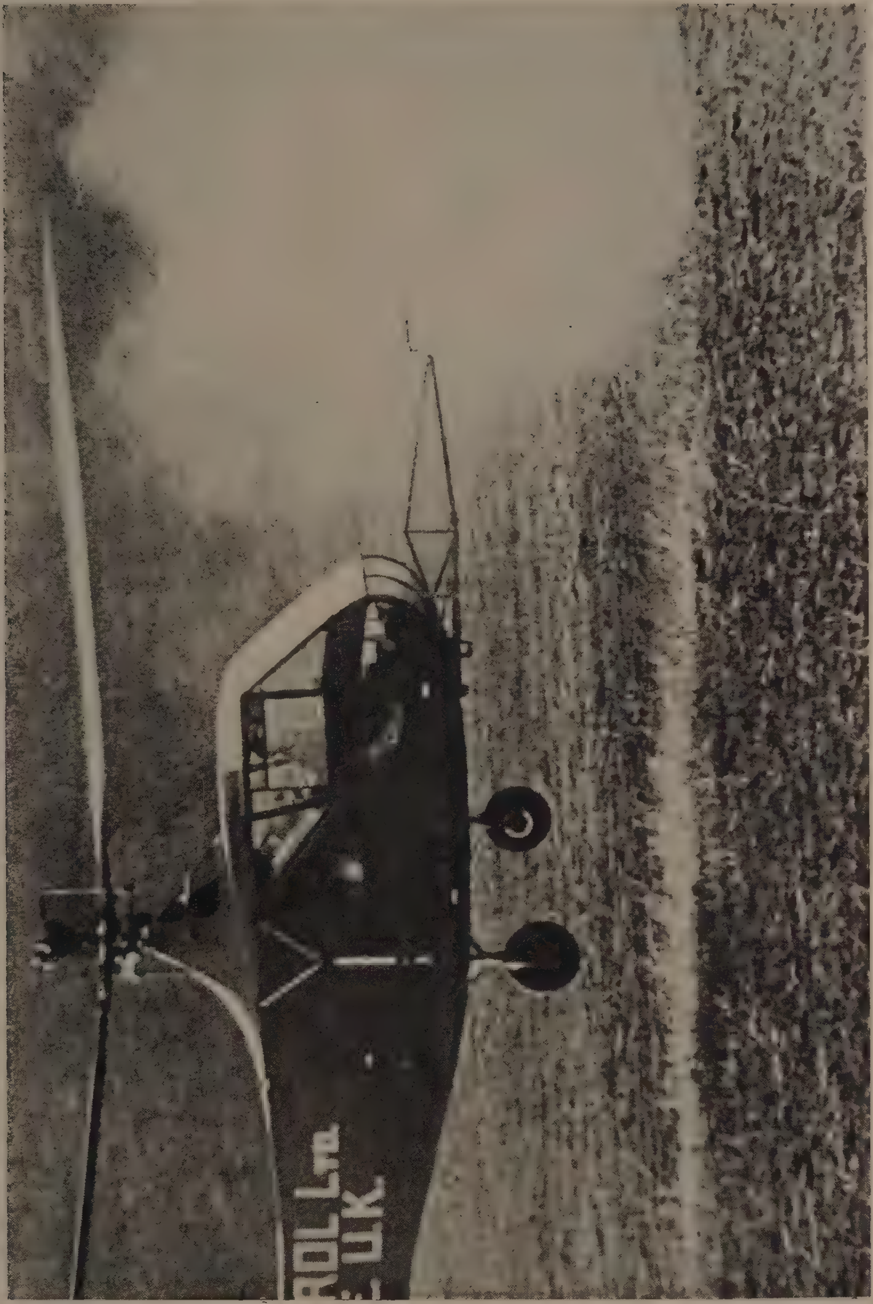
YR.4.B. helicopter with boom in "B" position.



YR.4.B. helicopter with boom in " C " position.



YR.4.B. helicopter with experimental spray bar discharging spray liquid a few feet above ground.



Spraying helicopter (YR.4.B.) flying at greater height above ground showing dispersal of spray liquid.

A SYNOPSIS OF THE GENUS *TRIBOLIUM* MACLEAY, WITH SOME REMARKS ON THE EVOLUTION OF ITS SPECIES-GROUPS (COLEOPTERA, TENEBRIONIDAE).

By H. E. Hinton, Ph.D.

Department of Entomology, British Museum (Natural History).

	PAGE
Introduction	13
Species-groups of *Tribolium*	15
Prototype of *Tribolium*	22
Relation of *Tribolium* to *Lyphia*	23
Generic diagnosis of *Tribolium*	25
Key to the species of *Tribolium*	26
Descriptions of new species	30

Introduction.

Several species of *Tribolium* are important primary and secondary pests of various kinds of cereal products and for this reason have long attracted the attention of entomologists. Some of the species are easily cultured in the laboratory and have therefore become favoured animals for experiments not particularly concerned with their rôle as pests. As a result of the two avenues of approach, a very impressive amount of information has now been accumulated concerning the habits and physiology of a few of the species.

In the course of revising the species of *Tribolium* of economic importance for the second volume of my monograph (Hinton, 1945) several interesting problems arose, the solution of which depended on a close study of all the species of *Tribolium* as well as on a study of related genera. The present synopsis is one result of this work. Notwithstanding the amount of information available on the habits and physiology of some of the species, there is practically no information on their relationships, and such problems as the country of origin of the pests that are now cosmopolitan have been almost entirely neglected. An attempt is here made to exploit the available morphological and distributional evidence in order to present a brief outline of the evolution of the various species-groups. Some of the conclusions are highly speculative and will have to be extended or partly altered when further species of *Tribolium* are discovered. The relation of the numbers of individuals in the different species that have been collected shows clearly that many more species of *Tribolium* are still to be discovered; and the discovery of these further species will reveal the extent to which the chief conclusions are correct.

An examination of the type of *Tribolium risbeci* Lepesme (1943) shows that it is a damaged specimen of the common *Alphitobius laevigatus* F. The genus *Aphanotus* is here sunk as a synonym of *Tribolium*, in which there are now nine species. A further 17 species, 1 subspecies, and 2 forms are described in this paper as follows :—

			Number of individuals of new forms.
		brevicornis species-group	
(1)	*Tribolium*	*brevicornis* (Lec.).	
(2)	,,	*linsleyi*, sp. n.	1
(3)	,,	*parallelus* (Casey).	
(4)	,,	*gebieni* Uyttenb.	
(5)	,,	*carinatum*, sp. n.	4
	,,	*carinatum dubium*, subsp. n.	3
		confusum species-group	
(6)	,,	*confusum* J. du V.	
(7)	,,	*anaphe*, sp. n.	11
(8)	,,	*destructor* Uyttenb.	
(9)	,,	*giganteum*, sp. n.	14
(10)	,,	*downesi*, sp. n.	7
(11)	,,	*semele*, sp. n.	2
(12)	,,	*sulmo*, sp. n.	2
(13)	,,	*indicum* Blair.	
	,,	*indicum* f. *seres*, *f. n.*	1
	,,	*indicum* f. *ares*, *f. n.*	1
(14)	,,	*thusa*, sp. n.	4
		alcine species-group	
(15)	,,	*alcine*, sp. n.	17
(16)	,,	*dolon*, sp. n.	1
(17)	,,	*ceto*, sp. n.	3
		castaneum species-group	
(18)	,,	*castaneum* (Herbst).	
(19)	,,	*madens* (Charp.).	
(20)	,,	*freemani*, sp. n.	1
(21)	,,	*waterhousei*, sp. n.	2
(22)	,,	*parki*, sp. n.	1
(23)	,,	*cylindricum*, sp. n.	17
(24)	,,	*politum*, sp. n.	1
		myrmecophilum species-group	
(25)	,,	*myrmecophilum* Lea.	
(26)	,,	*antennatum*, sp. n.	1
		Total number of individuals of new forms	94

In recent years there have been two revisions of the genus *Tribolium*, the first by Uyttenboogaart (1934) in which one new species, *T. destructor*, was described and the second by Good (1936) in which the biology of the four species known at that time to be of economic importance is summarised. The reader is referred to the revision by Good for historical notes on the taxonomy of the genus, which to save space are not repeated here. Types, cotypes, or topotypes of all the species have been seen except *T. gebieni*, which is known only from a description so inadequate that it has not been possible to place it in the key given later.

For the gift or loan of specimens I am indebted to Drs. P. Lepesme, E. G. Linsley, E. S. Ross, and Ray F. Smith. The illustrations of the whole insects were made by Mr. A. Smith.

The Species-groups.

Apart from the few species which have invaded stored products and have, therefore, been widely distributed by man in comparatively recent times, there is no evidence to suggest that the species of *Tribolium* are readily distributed under natural conditions. The fact that the Oriental, Madagascan, African, and American regions each have their characteristic species-groups is some evidence that the genus is at least as old as the Cretaceous after which S. American and the Indo-African continents had drifted apart. Against this supposition it might be argued that the present discontinuous distribution of *Tribolium* is but a remnant of a wider past distribution, and that sometime in the Tertiary, or perhaps even as late as the Quaternary glaciations, the species were wiped out in the Holarctic and survived only in tropical or subtropical regions. I have no reservations regarding the former theory, which involves accepting the broad outlines of Wegner's theory of continental drift.

Although the exact age and place of origin of the genus are at present largely matters of speculation, the country of origin of nearly all of the species, including those that are now cosmopolitan, can be positively determined. The species fall into five natural species-groups or subgenera as follows :—

(1) The *brevicornis* species-group (American. Subgenus *Aphanotus* Lec.).

This species-group ranges from Argentina to South-Western United States. In addition to *T. brevicornis* (Lec.), it includes *T. parallelus* (Casey), *T. linsleyi* sp. n., *T. carinatum* sp. n., and *T. gebieni* Uyttenb.

The average size of the species in this species-group is greater than that of any other in the genus although all are smaller than *T. giganteum* of the *confusum* species-group. The antenna has a feebly differentiated 5-segmented club like that of *T. confusum*. The margins of the head above the eyes are not distinctly ridged as in the *confusum* species-group, but in some species, *e.g. T. carinatum* sp. n., there is a feebly impressed line parallel to the hind part of the margin, so that the margin above the eyes appears to be very feebly ridged when viewed from some angles. The apex of the pronotum has a distinct marginal line on the side, which is continuous with the lateral marginal line, as shown in figs. 3 and 4. In all other species-groups of *Tribolium* the apex is unmargined, but the sides may curve inwards at the anterior angles and thus give the pronotum the appearance of having the extreme lateral part of the apex margined (fig. 2). The apical marginal line appears to be a primitive feature retained in the *brevicornis* species-group but lost in all others, and constitutes the most important distinguishing feature of the American species. Although the apical marginal line of the pronotum is absent in the closely related genus *Lyphia*, its presence is nearly universal in the more generalised DIAPERINI.

The elytra have the first nine intervals distinctly keeled. A general comparison of the DIAPERINI with and without carinate elytra leaves little room for doubt that, as among beetles generally, except possibly in the CUPEDIDAE, the possession of carinate elytra is a specialised feature. The prototype of *Tribolium* almost certainly had flat elytral intervals, as indeed have recent species in the *castaneum* species-group.

The secondary sexual characters of the male are unlike those of any other species-groups. In *T. carinatum* sp. n., the basal part of the ventral side of the hind femur is arcuately emarginate and clothed with long, erect hairs. The basal part of the middle femur is very slightly narrowed and also has some long, erect hairs. The secondary sexual characters of *T. brevicornis* are similar, but both the femora and tibiae of the middle and hind legs have distinct tubercles. Males of the remaining species are not available.

The *brevicornis* species-group is here regarded as the most primitive of the genus, chiefly on account of the margined apex of the pronotum. The elytra are strongly carinate and are as specialised as those of any other species-group, but, as is clear from the account given below, the elytra have independently acquired carinae at least once in each of three other species-groups; and for this reason relatively little importance can be attached to the possession of carinate elytra.

Of the *brevicornis* species-group, only *T. parallelus* is known to be associated with stored products, being up to now recorded only as a minor pest of stored cereals.

(2) The *confusum* species-group (African).

Nine species and two forms, probably representing two further species, belong to this group. The differences between this and the other four species-groups are mentioned when discussing the latter. The *confusum* species-group certainly appears to be more closely related to the *castaneum* than to the *brevicornis* species-groups when all external characters of the adults are taken into account. The more primitive members of the *confusum* and *castaneum* species-groups closely resemble each other both in general appearance and size, and both possess similar secondary sexual characters in the male. The closer relation of the *confusum* to the *castaneum* species-group is in accord with the theory held by some that the break between S. America and Africa occurred before the Indo-African break, so that the ancestors of the *brevicornis* species-group were the first to be isolated. This relationship might equally well be explained by the fact that, up to the late Cretaceous, India was separated from S. America by Africa and faunal exchanges between India and Africa were direct and near but those between India and S. America could only take place through Africa. There are three distinct sections in the *confusum* species-group, as follows :—

(*a*) The *confusum* section.

In addition to *T. confusum*, this section also includes *T. anaphe* sp. n. and *T. destructor* Uyttenb. These three species are very closely related, the smaller specimens of *T. anaphe* sp. n. being very close in general appearance to *T. confusum*, with which they have previously been confused, and the larger specimens closely resembling *T. destructor*. The males of *T. destructor* lack the sub-basal pit and associated brush of hairs present in the other two species on the ventral side of the front femur.

The country of origin of *T. confusum* and *T. destructor* has long been a subject for speculation. The type locality of *T. confusum* is France, that of *T. destructor* Germany. There can be little doubt that the two species are Ethiopian. This is not only indicated by their very close relationship to *T. anaphe* sp. n. of tropical Africa but also by the fact that all other members of their species-group are Ethiopian. *T. destructor* was described in 1933 from Germany and the Netherlands, and it has since been found to be a pest in North America and Africa. A specimen collected in Abyssinia and presented to the British Museum by J. C. Browning in 1863 has been examined and another in the Paris Museum with the data : Abyssinia, 1850 (*Schimper*). These specimens of *T. destructor* were thus collected some 70 and 83 years respectively before the species was found in Europe and described ; and on these grounds alone it may, therefore, be assumed with a considerable measure of confidence that the species is a native of Africa.

T. confusum is widely distributed in Africa where it has been found out-of-doors, but this fact cannot be cited as evidence of its African origin, since most of the cosmopolitan pests of stored products, including species of *Tribolium*, can and do maintain themselves out-of-doors where the temperature is suitable and the necessary food is available. For instance, *T. confusum* itself occurs under bark and in the nests of *Anthophora*, *Osmia*, and other bees in California and elsewhere in North America where everyone agrees it is introduced. The evidence that *T.*

confusum is an African species rests, as already stated, on its relationship to *T. anaphe* sp. n. and *T. destructor* and on the fact that it belongs to an apparently exclusively African species-group. Andres (1931) records the occurrence of a species of *Tribolium* in a pharaonic tomb of the VI Dynasty (*ca.* 2,500 B.C.). This *Tribolium* was found in a jar which probably contained grains of flour. The specimens had the legs and antennae missing. Andres was unable to decide if they were *T. confusum* or *T. castaneum*, as he was unaware of the difference between the two species as regards the division of the eyes and the margins above the eyes. On indirect evidence, however, it appears fairly certain that the species was *T. confusum*, as at this period or before there was little if any communication between the Nile Valley and Southern Arabia ; and it is hardly conceivable that any *Tribolium* could have been not only carried from India or neighbouring regions into Persia by the Sumerians but then later transported into Southern Arabia.

(*b*) The *giganteum* section.

In this section only *T. giganteum* sp. n. is included. It is much larger than any other known species, being 10–12 mm. long, whereas the next largest species in the genus is not more than 7 mm. Its antennae are unique in having a feebly differentiated 7-segmented club. Except for its great size and many segmented antennal club, it would have been included in the *confusum* section to which it is otherwise very closely related. Only two other species in the genus, *T. alcine* sp. n. and *T. confusum*, have sub-basal pits and brushes of hairs on the front and middle femora. In both the latter species there is a slight sub-basal brush (only a few hairs) on the hind femora, whereas in *T. giganteum* there is a small but distinct tubercle in the homologous position. The presence of a tubercle on the hind femora of the male suggests a retention of the kind of secondary sexual characters found in the *brevicornis* species-group, *e.g.* in *T. brevicornis*, but little emphasis can be placed on a general resemblance of this sort, since there is no independent evidence that the *brevicornis* species-group has retained the primitive secondary sexual characters of the genus ; and it is possible that the *confusum-alcine-castaneum-myrmecophilum* species-groups on one hand and the *brevicornis* on the other have evolved from a prototype with secondary sexual characters unlike those of any recent species-group.

(*c*) The *downesi* section.

In this, the largest section, are also included *T. semele* sp. n., *T. sulmo* sp. n., *T. indicum* Blair with its two new forms, and *T. thusa* sp. n. The important structural differences between this and the *confusum* section clearly point in nearly every case to the higher specialisation of the *downesi* section : the antennae have a much more abrupt and clearly differentiated club owing to a much greater difference in breadth between the sixth and seventh segments ; the punctures of the pronotum are relatively coarser, denser, and in most species more uniform in size and distribution ; the elytra are more strongly carinate, the second interval always being carinate and the first usually so ; and the usual secondary sexual characters of the male are lost. The eyes of the *downesi* section are larger, less completely divided, and approach each other more closely beneath the head ; and in this respect only it may be less specialised than the *confusum* section. Since nothing is yet known of the physiology of the *downesi* section, it cannot be said that the *confusum* section is more specialised in this respect, *i.e.* in the toleration of low moisture contents, low relative humidities, and so on.

In *Tribolium*, as in most beetles of whatever family, the pronotal punctures are coarser and denser at the sides than on the disk. In *Tribolium*, as already stated, the direction of evolution is in many sections clearly towards coarser, denser, and more uniformly distributed pronotal punctures. By using such criteria, it is possible to discriminate tentatively between the species in the *downesi* section as regards their degree of specialisation. The two most primitive species are probably *T.*

downesi sp. n. and *T. semele* sp. n., which still have the lateral pronotal punctures very different from the discal. *T. sulmo*, sp. n. is probably derived from an ancestor of this kind and is intermediate between the *downesi-semele* group and the *indicum-thusa* group. The two latter species are clearly the most specialised, as is shown by the evenly distributed pronotal punctures and the acquisition of pronotal tubercles. It is not, of course, suggested that the recent species are derived from each other.

(3) The *alcine* species-group (Madagascan).

The three Madagascan species, *T. alcine* sp. n., *T. dolon* sp. n., and *T. ceto* sp. n. belong to a species-group which in some important particulars is intermediate between the *castaneum* and *confusum* species-groups. The species have the head structure, *i.e.* margins above eyes not ridged, of *castaneum* and its allies, but two of the species have the characteristic 5-segmented antennal club of *confusum* and its relatives, whereas in the third, *T. dolon* sp. n., the antenna is evenly broadened to the apex (as in many species of *Palorus*) and there is no distinct club.

The differences between the species are as great or nearly as great as those found within the *confusum* species-group. Since there is no evidence for believing that the rate of evolution of *Tribolium* is any greater in Madagascar than in Africa, the differences between the species would seem to indicate that the *alcine* and *confusum* species-groups have been evolving for similar periods.

It could be supposed that the Madagascan species are the result of at least three separate invasions from E. Africa but for the fact that this would involve assuming that in each instance the species entering Madagascar has independently lost the ridges over the eyes. Nor does it appear in the least probable that the *alcine* species-group is derived from a single African species without ridges over the eyes entering Madagascar as late as the Oligocene by the land connection sometimes postulated for this period. If such a theory were correct, it would probably be necessary to assume that all the known African species are derived from a single species as recently as the Oligocene, which is most unlikely in view of the great structural differences between the African species, differences at least as great as those between the species in the American and possibly also Oriental species-groups which have been evolving since the Cretaceous. Furthermore, it must be noted that a Madagascan-E. African land connection in the Oligocene (or Miocene) has been postulated to account for the presence in Madagascar of lemurs, centetids, viverrids, and some other animals but has not yet been in any way conclusively demonstrated. Matthew (1915), says of Madagascar : " The characters of the mammalian fauna, both negative and positive, practically exclude the theory of land connections in the Tertiary." Du Toit (1937) cites the occurrence of the pigmy hippotamus in Madagascar as evidence for an Oligocene land connection with Africa, but the hippotomai are not earlier than the Pliocene and if they entered Madagascar other than by swimming it would still be necessary to account for the absence on the island of many groups of mammals that might have been expected to enter Madagascar by the same land connection.

Evidence for land connections from the distribution of mammals and other advanced vertebrates becomes increasingly reliable as we proceed from the Eocene to recent times, but for earlier periods can hardly be compared with that derived from the distribution of invertebrates. Before the end of the Eocene the continents were in much their present positions, and the evidence of Matthew and others for the Holarctic dispersal of Tertiary mammals cannot be, as is often done, cited as evidence against the theory of continental drift.

As already stated, it seems likely that the rate of evolution—except under special circumstances, as shown for the *myrmecophilum* species-group—is roughly the same within the genus. If this is so, the evolution of the Madagascan species is likely to have commenced in the Upper Cretaceous, since the differences in structure between the three are qualitatively equivalent to the differences between species of species-groups that are undoubtedly of Cretaceous origin. The opposed coasts of E. Africa and Madagascar are bordered by marine Jurassic deposits, and nearly all authors are agreed that since the end of the Trias there have been no land connections between Madagascar and Africa except possibly in the Oligocene. For this reason, and those stated above, it is believed that the entry of *Tribolium* into Madagascar may be explained in two ways, of which the first seems more likely : (1) the prototype of the *alcine* species-group entered Madagascar in the Upper Cretaceous from India at a time when the Oriental species had a 5-segmented antennal club, and that all the Oriental species are derived from a single species which evolved a 3-segmented antennal club in the Upper Cretaceous or early Tertiary ; or (2) the prototype of the *alcine* species-group entered Madagascar from Africa as a result of an accidental transportation across the relatively narrow sea which separated the two in the Upper Cretaceous or early Eocene at a time when at least some African species had no ridges above the eyes.

(4) The *castaneum* species-group (Indo-Australian. Subgenus *Tribolium* Macleay).

In this group are included *T. castaneum*, *T. madens*, *T. freemani* sp. n., *T. cylindricum* sp. n., and *T. politum* sp. n. from the Oriental Region and *T. waterhousei* sp. n. and *T. parki* sp. n. from the Australian Region. It is fairly certain that *T. myrmecophilum* and *T. antennatum* sp. n. should be included in this species-group rather than in a separate one, as has been done here largely for the sake of convenience.

The *castaneum* species-group differs from all others in its very distinct and abrupt 3-segmented antennal club. In addition, it may be distinguished from the *brevicornis* species-group by having the apex of the pronotum unmargined and from the *confusum* species-group by not having the margins above the eyes ridged. Of all the species-groups it is the most variable and has already split into a number of very distinct secondary species-groups or sections. It is of some interest to note that the great differentiation of the species occurs in just that part of the Oriental Region which has undergone, in many respects, the greatest physical changes. The eastern part of the Oriental Region between the present Asiatic mainland and the Australian Region has undergone immense changes in its available land mass in the early Quaternary with the result that populations of *Tribolium* were often completely isolated on diverse kinds of islands which must have undergone considerable changes in their fauna and flora as they were in a fairly short time in the Pleistocene or a little later reduced to their present areas by transgression of the sea.

(*a*) The *castaneum* section (Indian, except *T. madens ?*).

This section also includes *T. madens* (Charp.) and *T. freemani* sp. n., all three species being very closely related. They differ from other species in the *castaneum* species-group in having some of the elytral intervals distinctly ridged (the elytra of an occasional specimen of *T. castaneum* is not distinctly ridged) and in having a distinct reticulate microsculpture between the punctures of the dorsal surface.

Except in the case of *T. madens*, there is no doubt as to the country of origin of the species of *Tribolium* which have become pests of stored products and have been widely distributed by man. The country of origin of *T. madens* is in some doubt. It was first found in a beehive in Silesia in 1825 and has since been found out-of-doors and in stored products in various European countries including Austria and France. It has not, so far as I am aware, ever been introduced into the British Isles. It was first recorded in the United States in 1866, where it has now successfully established itself both out-of-doors and in mills and warehouses. It has also been found in Egypt. It has clearly been introduced into both the United States and Egypt. It is difficult to believe that it is a native of Northern or Central Europe in view of its extremely close relationship to two indisputably Oriental species. The latter fact suggests that it too is an Oriental species introduced into Europe early in the last century by commerce. As against this supposition, it must be stated that it has not yet been reported from any part of the Oriental Region either in stored products or out-of-doors, although it must be remembered that this vast region is by no means thoroughly explored so far as small beetles are concerned.

(*b*) The *waterhousei* section (Australian).

To this section belong the two closely related species, *T. waterhousei* sp. n. and *T. parki* sp. n. The *waterhousei* section is much more closely related to the *castaneum* section than are either of the *cylindricum* or *politum* sections. The *waterhousei* section differs from the *castaneum* chiefly in having the intervals of the elytra only feebly instead of distinctly carinate and in having the dorsal surface smooth and polished instead of reticulate between the punctures. The external secondary sexual characters of both sections are similar, *i.e.* the male has a sub-basal pit and associated brush of hairs on the ventral side of the front femur.

There would appear to have been only two possible times when the *waterhousei* section could have entered Australia: (1) from America in or before the Eocene, *i.e.* some 70 million years or more ago, or (2) from the Oriental Region in the Pleistocene during one of the northern glaciations which lowered the sea-level sufficiently to bring the dry-land area of the Asiatic and Australian continents to less than 100 miles of each other. The first possibility is extremely unlikely, and would mean that the *castaneum* species-group, as here defined, is polyphyletic. If true, the *waterhousei* section should be nearer morphologically to American than to the Oriental species of *Tribolium*, but the reverse is the case. Furthermore, the very close similarity between the *waterhousei* and *castaneum* sections would have to be explained as due to convergence of parallelism of a very unlikely kind, since the climatic history of the Australian continent as it drifted northwards to Asia must have been very different indeed from that of the Oriental Region.

(*c*) The *cylindricum* section (Malay Peninsula, East Indies).

In this section only *T. cylindricum* sp. n. is included. It differs from all other members of its own and other species-groups of *Tribolium* in its sub-cylindrical body and its narrow prothorax, which is approximately as long as broad. Apart from this somewhat striking change in body form, it is close to the *castaneum* section. The elytral intervals are indistinctly keeled and the male has a sub-basal pit and associated brush of hairs on the ventral side of the front femur, as in the *castaneum* section.

T. cylindricum has been found in the Malay Peninsula from Kuala Lumpur to Singapore. Its occurrence so far away as on Batchian in the Moluccas and its apparent absence on intermediate islands might suggest transportation by man but for the fact that the beetles living beneath bark in the East Indies have scarcely been investigated. In this connection it should be noted that various writers have drawn attention to the fact that the Pleistocene glaciations in northern latitudes had the effect of lowering the sea level from 100 to 200 metres. The 100 and 200 m. depth lines are close together, and a drop in the sea level of this order had the effect of uniting the Malay Peninsula with Borneo in a continuous land mass. The eastern tip of Borneo is only about 600 miles from the Moluccas, and this 600-mile stretch is partly bridged by the Celebes and other islands, so that when the level of the sea was lowest in the Pleistocene there was nowhere a stretch of sea of more than 100 miles between the Asiatic mainland and Batchian.

(*d*) The *politum* section (Riouw Archipelago).

T. politum sp. n. must be placed in a section of its own in the *castaneum* species-group. The nearly impunctate dorsal surface and the absence of striae and strial punctures on the elytra are conspicuous features which will serve to distinguish it from any other species of *Tribolium*. If, as I think, the prototype of *Tribolium* had distinctly striate-punctate elytra with more or less flat intervals, *T. politum* has departed perhaps more widely from such a prototype than any other member of the *castaneum* species-group. In all other species-group it is clear that the direction of evolution is towards developing more strongly carinate elytra and increasing the relative size and density of the punctures of the dorsal and ventral surfaces. In respect of these characters, *T. politum* has evolved in the opposite direction and represents an end line to evolution. No other known species in the genus can be derived from the immediate ancestors of *T. politum*.

(5) The *myrmecophilum* species-group (Australian. Subgenus *Leanum* Uyttenb.).

This species-group includes only two species, *T. myrmecophilum* Lea and *T. antennatum* sp. n. The absence of a basal marginal line on the prothorax and the narrow apical segment of the antennal club, which is distinctly narrower than the penultimate segment, are the two principal distinguishing features of this group, neither character being possessed by any other species of the genus.[1] The narrow apical segment of the antenna, particularly that of *T. antennatum* sp. n., is very similar to that of *Latheticus*. The antennal club (fig. 33) is probably to be regarded as 3-segmented, but the breadth of the two preceding segments is sufficient to make it very difficult to decide whether it is 3- or 5-segmented. Males and females of *T. myrmecophilum* have been examined, and there appear to be no external secondary sexual characters. Certainly the males have not the sub-basal ventral pit and associated brush of hairs which are present in the males of most species of the *castaneum* species-group.

T. myrmecophilum is known only from nests of ants (*Iridomyrmex nitidus* Mayr). The strongly dilated and slightly flattened antennae of *T. antennatum* are probably the result of a similar environment. The flattening and dilation of the antennal segments, particularly of the apical ones, is a characteristic morphological modification undergone by a number of different groups of beetles, *e.g.*, some CARABIDAE, CLAVIGERIDAE, PTINIDAE, etc., as they become adapted to living in ants' nests. The much more flattened antennae of *T. antennatum* and its carinate instead of flat elytral intervals clearly show that it is more specialised than *T. myrmecophilum*.

The *myrmecophilum* species-group is more nearly related to the *castaneum* species-group than to any other. It possesses no especial affinities with the American species, as might be expected if it had entered Australia in the Eocene when Australia was connected by way of Antarctica to South America. This fact, considered together with its evident relationship to the *castaneum* species-group, strongly suggests that the *myrmecophilum* species-group has entered the Australian Region from the Oriental in comparatively recent times, that is, in the late Tertiary or early Quarternary. If this theory is correct and the immediate ancestors of the *myrmecophilum* species-group have indeed entered Australia only recently, it is still necessary to account for the relatively great morphological differences that exist between the *myrmecophilum* and *castaneum* species-group. These differences are, for instance, greater than those that exist between any two other species-groups in the genus, notwithstanding the fact that the other species-groups have apparently been separated since the Cretaceous or early Eocene. The great morphological differences that exist between the *myrmecophilum* species-group and its nearest relatives are almost certainly due to the invasion of ants' nests by the former. Comparisons made between groups of species that have entered ants' nests and their close relatives which have not done so show that the rate of speciation and morphological differentiation is vastly greater amongst the myrmecophilous species which are tolerated or cared for by the ants. This fact may be observed in any family of beetles which has myrmecophilous species.

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[1] The apical antennal segment of *T. dolon* sp. n. is somewhat narrowed but in much less degree than in the *myrmecophilum* species-group, where it is also flattened.

The view that the *myrmecophilum* species-group has recently entered Australia is further supported by the fact that relatively unmodified members of the *castaneum* species-group are found to-day in that region, *i.e.*, *T. waterhousei* sp. n. and *T. parki* sp. n. The occurrence of these members of the *castaneum* species-group in the Australian Region is good evidence that the Oriental species of *Tribolium* have been able to invade the Australian Region.

From what has been said in the preceding paragraphs, it is evident that from a phylogenetic point of view the *myrmecophilum* species-group has not the same status as the *brevicornis*, *castaneum*, and other species-groups, but should be regarded as no more than a section of the *castaneum* species-group despite its greatly modified antennae and prothorax and the loss of the usual male secondary sexual characters.

Prototype of *Tribolium*.

From the foregoing discussion of the various species-groups, it is clear that it is possible to determine with a certain degree of probability some of the more important morphological characteristics of the primitive *Tribolium* from which the recent species-groups arose. It is considered that the primitive *Tribolium* possessed the following combination of characters :—

(1) Body moderately convex with the sides subparallel.

(2) Punctures of dorsal and ventral surfaces moderately fine and moderately sparse, *e.g.*, as in the *waterhousei* section of the *castaneum* species-group.

(3) Area between punctures of dorsal and ventral surfaces probably without an evident microsculpture.

(4) Antenna with segments progressively broadened towards apex or with a feebly differentiated 5-segmented club, the difference between the breadth of sixth and seventh segments being slight.

(5) Eyes only partly divided.

(6) Margins above eyes not ridged.

(7) Pronotum with apex margined, at least at sides, and sides and base completely margined.

(8) Elytra striate-punctate. Intervals of elytra flat and without trace of carinae or keels.

(9) Elytra with epipleura incomplete at apex, as in all recent species-groups.

Of the characters listed, " (2) " and " (3) " are much less certain than the remaining, and it is with considerable hesitation that they are included. It is difficult to believe that the common ancestor of the recent species-groups did not possess " (1) " and " (4) " to " (9) " of the above list.

The relationships of the species-groups to each other, as conceived in this paper, are diagrammatically illustrated in fig. 1. I prefer to keep the five species-groups in the same genus, but assuming fig. 1 to be objectively correct four other taxonomic possibilities—which reflect differences of opinion but not of fact—are open to us, namely :—

(1) A genus may be erected for each section of each species-group.

(2) Five genera may be employed, one for each species-group.

(3) Four generic names may be used, one for the *brevicornis* species-group, one for the *castaneum-myrmecophilum* species-groups, and one for each of the two other species-groups.

(4) Two genera may be kept, one for the *brevicornis* species-group and one to include the four remaining species-groups, as is the present practice.

These alternative classifications would all be based on the relative times of evolution and would be phylogenetically sound. A classification based essentially on the rate of evolution rather than on the relative time of evolution might in some cases be commended from a practical point of view but would fail to give a correct view of the phylogeny. For instance, the *myrmecophilum* species-group (in reality only a section of the *castaneum* species-group) differs strikingly in morphological characters from the remaining species-groups. These differences are so great and so conspicuous that a taxonomist unaware of the phylogeny of the group would be completely justified in erecting a genus for the *myrmecophilum* species-group and retaining all other species-groups in a single genus, but in so doing the latter genus would inevitably be polyphyletic.

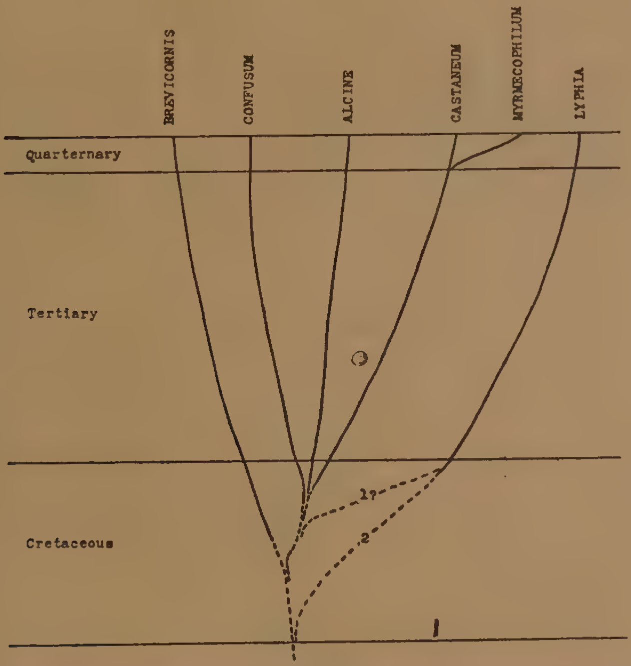


Fig. 1.—Phylogenetic diagram illustrating the relations of the species-groups of *Tribolium* to each other and to *Lyphia*. If " 1 " is correct, *Lyphia* should be included in the genus *Tribolium*, but it is here assumed that " 2 " is correct.

Relation of *Tribolium* to *Lyphia*.

If the common ancestor of the recent species-groups of *Tribolium* possessed the characters listed in the previous section, it apparently could equally well have been the ancestor of the recent genus *Lyphia*. There are some 18 known species of *Lyphia*. These occur in the Mediterranean and Ethiopian Regions, in Madagascar, throughout the Oriental Region, and in both New Guinea and Australia. Until now they have not been found in South America. *Lyphia* differs from *Tribolium* in having the eyes much more prominent than the sides of the front and in having a well defined 4- instead of 7-, 5-, or 3-segmented antennal club. The species are nearly always more cylindrical than *Tribolium*, as defined in this paper, and the elytra are usually more or less confusedly punctate and never carinate, as is so frequently the case in *Tribolium*. The pronotum has the apex unmargined, as in all species-groups of *Tribolium* except the *brevicornis*.

Although there are at present no records of *Lyphia* in South America, it should be remembered that S. America is, from an entomological point of view, the least known of the larger continents. If, however, *Lyphia* is actually absent from S. America, as now appears to be the case, and never did occur there, the genus probably originated on the Indo-African block after separation of the latter from South America. This would mean that the immediate ancestors of the species now found in New Guinea and Australia have entered Australasia from the Oriental Region only relatively recently, probably in the Pleistocene. The Australian species are so intimately related to the Oriental that there can be no objection to this on morphological grounds.

Most of the individual characters which distinguish *Lyphia* from *Tribolium* are the kind of characters which distinguish species-groups or even sections of species-groups of *Tribolium* from each other. Among such characters are the structure of the elytra, the general body shape, and the 4-segmented antennal club. The difference of greatest significance between the two genera may lie in the structure of the head. In *Tribolium* the sides of the front are more prominent than the eyes (in *T. ceto* sp. n. the eyes are slightly more prominent), whereas in *Lyphia* the eyes are much more prominent than the sides. Whether *Lyphia* is accorded generic rank or is regarded as no more than a species-group of *Tribolium* depends not only on what value may be placed on this difference in head structure and the 4-segmented antennal club but on the value given to the different combination of characters possessed by *Lyphia*, since it is sometimes the case that two different but closely related groups may differ constantly in no single character but in different ways in which the total series of characters are combined.

The difference in the relative prominence of the sides of the front and the eyes places *Lyphia* in the DIAPERINI and *Tribolium* in the ULOMINI (the two categories are often given subfamily rank) in any key to the tribes of the TENEBRIONIDAE published since Lacordaire's key of 1859. While this is hardly the place to enter into a discussion of the TENEBRIONIDAE as a whole, it is necessary to point out that the prominence of the eyes, which according to most writers separates the DIAPERINI from the ULOMINI, results in an unnatural aggregate of genera when used irrespective of other characters. Among other defects, a division based on the structure of the head places the female of *Gnathocerus maxillosus* F. in the DIAPERINI whereas its male and both sexes of *G. cornutus* F. remain in the ULOMINI. Recent studies of the larvae do not support the separation of the ULOMINI from the DIAPERINI, although it must be admitted that relatively few larvae have been studied. However, despite the fact that the tribes ULOMINI and DIAPERINI, as at present constituted, cannot be maintained as distinct, it is clear that a large group of genera in the DIAPERINI is monophyletic, and, although varying considerably in many other ways, always has the eyes more prominent than the sides of the front. In other words, the character of having the eyes more prominent than the sides of the front appears in some sections of the family to be of more than generic rank. For these reasons it is difficult without further information to postulate a common ancestor for *Lyphia* and the *alcine-confusum-castaneum* species-group after the separation of the *brevicornis* species-group. If this should later prove to be the case when further facts are brought to light, *Lyphia* must either be incorporated in *Tribolium*, or, if maintained as a distinct genus, there is no alternative but to give generic rank to the *brevicornis* species-group. It is, however, possible that many of the similarities between *Lyphia* and the *alcine-confusum-castaneum* species-groups are due to convergence, and that the common ancestor of the two anti-dates the separation of the *brevicornis* species-group. I have, without any great conviction, dealt with *Tribolium* as if the latter hypothesis were correct.

Tribolium ceto sp. n. of Madagascar has the eyes very slightly more prominent than the sides of the front, but in its strongly carinate elytra, 5-segmented antennal club, etc., it is clearly a *Tribolium*. Although its eyes are not as prominent as in *Lyphia*, the possibility that a species of *Tribolium* will be discovered with eyes as prominent cannot be excluded; and in such a case the two genera would have to be distinguished on their different combination of characters, assuming that species of *Tribolium* are found with a 4-segmented antennal club.

Tribolium Macleay.

1825. *Tribolium* Macleay, Annulosa javanica, p. 47.

1862. *Aphanotus* Leconte, Class. Col. N. Amer., p. 233 (*new synonymy*).

GENERIC DIAGNOSIS:

Species 2–12 mm. long. Body moderately depressed to moderately strongly convex with sides parallel or subparallel. Dorsal surface with or without distinct hairs. Cuticle pale reddish or yellowish brown to black. *Head* never tuberculate. Antennae 11-segmented with a feebly or sharply differentiated 5-, 3-, or 7-segmented club, rarely gradually broadened to apex and without a distinct club; second segment usually distinctly broader than third and shorter than, to nearly as long as, the latter. Eyes partly to completely divided by sides of front. Clypeus with anterior margin truncate to moderately emarginate. Maxilla with apical segment of palp sub-cylindrical. *Pronotum* rarely as long as broad, usually distinctly broader than long. Sides more or less rounded and completely margined; apex not margined or (*brevicornis* species-group) with a marginal line which is usually confined to sides; base completely margined except in the *myrmecophilum* species-group. Elytra striate-punctate except in *T. politum* sp. n. and with intervals flat or feebly to distinctly carinate; epipleura vertical and indistinct near apex. *Prosternum* with apex of process flat or (*brevicornis* species-group) moderately convex and not or only slightly projecting behind hind margin of hypopleura. Mesosternum shallowly to deeply, triangularly emarginate. Abdomen with first two sternites completely margined at sides, third sternite partly or completely margined, fourth sternite usually partly or completely margined but sometimes without a marginal line, and fifth usually with a marginal line which is nearly complete to base, or without a marginal line. *Legs* with front coxae scarcely noticeably transverse; middle coxal cavities narrowly open laterally to metasternal epimeron. Tibiae slightly flattened at apices; front tibia with apex feebly to moderately dilated and outer (dorsal) edge smooth or feebly spinose, never evidently denticulate; hind tarsi with basal segment slightly shorter to slightly longer than the combined length of the two following segments.

GENOTYPE: *Colydium castaneum* Herbst (1797) (cited by Lucas, 1855).

COMPARATIVE NOTES: *Tribolium* is more nearly related to *Lyphia* than to any other genus. From *Lyphia* it may be distinguished at once by having the sides of the front of the head distinctly less prominent than the eyes, except in *T. ceto* sp. n. All known species of *Lyphia* have an abrupt 4-segmented antennal club, whereas no species of *Tribolium* has yet been discovered with a 4-segmented club. The genus *Platybolium* Blair is said to be closely related to *Tribolium* by Blair (1938, Ent. mon. Mag., **74,** p. 222) who says, " The very stout antennae suggest ULOMINI, but the structure of the head and legs forbids this association, as also any with *Alphitobius*. In spite of its broader flatter form the carinate intervals of the elytra are similar to those of *Tribolium* Macl. and *Aphanotus* Lec. In Seidlitz' key . . . it would run down to *Tribolium*, from which, however, it differs, apart from its broader form, in the broad side margins of the thorax, stout antennae and the stouter, somewhat arcuate tibiae." *Platybolium* is in no way related to *Tribolium*, but is clearly a specialised *Alphitobius*, and like the latter genus has the elytral epipleura horizontal and complete to the apex instead of vertical and indistinct on the apical region, as in *Tribolium* and *Lyphia*.

KEY TO THE SPECIES OF *Tribolium*.

1. Pronotum with apex distinctly margined (figs. 3–4) except usually on middle third to three-fifths, the apical marginal line being continuous with the lateral (*brevicornis* species-group)........2

— Pronotum with apex unmargined (fig. 2), but sometimes with sides curved inwards at apex so that at first sight the extreme lateral part of the apex may appear to be margined........6

2. Head with antero-ventral margin opposite base of maxilla produced to form a long, stout, very conspicuous tooth. Eye with greatest diameter of dorsal part only half of length of head in front of eyes. Abdomen with lateral marginal line of second sternite incomplete behind; fifth sternite with marginal line present only near base or, if complete, very close to apex. Male with a row of tubercles on ventral sides of both femora and tibiae of middle and hind legs. (Eyes completely divided. Punctures of fronto-clypeal region obovate, nearly round. Pronotum with anterior angles, when seen from above, less than twice as thickly margined as sides.) Length, 5–7 mm. California........*brevicornis* (Leconte, 1859)

— Head with antero-ventral margin opposite base of maxilla not or only indistinctly toothed. Eye with greatest diameter of dorsal part about equal to length of head in front of eyes. Abdomen with lateral marginal line of second sternite complete; fifth sternite with marginal line complete, often distant from apex. Male (except *T. linsleyi* & *T. parallelus* ?) without tubercles on ventral sides of femora and tibiae of middle and hind legs........3

3. Pronotum with anterior angles (fig. 4), when seen from above, about four times as thickly margined as sides. (Eyes completely divided. Fifth abdominal sternite with marginal line separated from apex by more than the breadth of an eye facet.) Length, 5·1–6·5 mm. Western North America........ *parallelus* (Casey, 1890)

— Pronotum with anterior angles (fig. 3), when seen from above, less than twice as thickly margined as sides........4

4. Eyes separated beneath head by only twice the greatest diameter of the ventral part of an eye. Abdomen with marginal line of fifth sternite separated from apex by about twice the breadth of an eye facet. (Eye with narrowest part, where divided by side of front, as broad as three facets.) Length, 6·5 mm. Mexico........*linsleyi* **sp. n.**

— Eyes separated beneath head by 2·5 to 3·2 times the greatest diameter of the ventral part of an eye. Abdomen with marginal line of fifth sternite nearly contiguous with apex. Length, 4·3–5·5 mm........5

5. Eye completely divided or with narrowest part, where divided by side of front, no broader than a single facet. Argentina (La Rioja Prov.).... *carinatum* **sp. n.**

— Eye with narrowest part, where divided by side of front, as broad as two or three facets. Argentina (Santiago del Estero).......*carinatum dubium* **subsp. n.**

6. Pronotum with middle two-thirds of base not margined. (*myrmecophilum* species-group)........7

— Pronotum with base completely and distinctly margined........8

7. Elytral intervals two to nine each with a fine but prominent carina. Antenna with club strongly flattened. Eye with narrowest part, where divided by side of front, consisting of four rows of facets. Metasternal disk with punctures as coarse as facets of eyes. Abdomen without an apical marginal line on fifth sternite. Length, 3·5 mm. Queensland........................*antennatum* **sp. n.**

— Elytral intervals flat or feebly convex, without trace of carinae. Antenna with club hardly more flattened than usual in the genus. Eye with narrowest part, where divided by side of front, consisting of only three rows of facets. Metasternal disk with punctures distinctly finer than facets of eyes. Abdomen with a very distinct apical marginal line on fifth sternite. Length, 4·0–4·5 mm. Victoria...*myrmecophilum* Lea (1904)

8. Head with margins above eyes elevated to form a distinct ridge. Antennal club of five or more segments. (*confusum* species-group)...............................9

— Head with margins above eyes not elevated. Antennal club of three or five segments..17

9. Species 10–12 mm. long. Antenna with a 7-segmented club. Male with a small but distinct sub-basal tubercle on ventral side of hind femora. (Cuticle black or nearly so. Clypeus with a broad and moderately deep, truncate emargination. Eye with narrowest part, where divided by side of front, consisting of a single facet. Elytra with fifth and sixth intervals strongly carinate but other intervals flat or only inconspicuously ridged.) Kenya...... *giganteum* **sp. n.**

— Species less than 6 mm. long. Antenna with a feebly to strongly differentiated 5-segmented club. Male without tubercles on ventral side of hind femora...10

10. Elytra with first and second intervals flat or at most carinate only on apex. Antenna with club only feebly differentiated, the seventh antennal segment being less than half again as broad as the sixth. Eye with narrowest part consisting of three or fewer facets. (Pronotum, fig. 21, with round to obovate punctures irregularly distributed, some punctures being separated by one or two, or, occasionally, more diameters. Abdomen with punctures of sides of sternites distinctly coarser and usually denser than on disk.)....................11

— Elytra with second interval sharply carinate from base to apex and sometimes with the first (sutural) interval also carinate from base to apex. Antenna with club sharply differentiated, the seventh segment being more than half again as broad as the sixth. Eye with narrowest part, where divided by side of front, consisting of three or more than three facets.........................13

11. Eye with narrowest part, where divided by side of front, no broader than a facet; eyes separated beneath head by three or nearly three times the transverse diameter of the ventral part of an eye. Length, 2·6–4·4 mm. Cosmopolitan.......................................*confusum* Jacquelin Du Val (1868)

— Eye with narrowest part, where divided by side of front, as broad as two or three facets together, but if only as broad as a single facet (some specimens of *T. destructor*), the body is 4·5–5·5 mm. long with cuticle very dark reddish brown to black; eyes separated beneath head by less than two to very little more than two times the transverse or greatest diameter of ventral part of an eye 12

12. Middle of head with punctures smaller than facets of eyes, usually round but sometimes feebly oval, and with none or very few of the punctures longitudinally confluent; surface between punctures with a reticulate microsculpture. Eye with narrowest part, where divided by side of front, as broad as two or three facets. Cuticle moderately dark reddish. Male with a sub-basal pit and brush of hairs on ventral side of front femora; male genitalia (figs. 10–11) with parameres abruptly narrowed before apex. Length, 3·59–5·08 mm. Nigeria, French Sudan, Congo, Uganda, Mashonaland............*anaphe* **sp. n.**

— Middle of front with punctures usually as long as facets of eyes, oval, and frequently longitudinally confluent so that surface appears longitudinally strigose; surface between punctures smooth or nearly so. Eye with narrowest part, where divided by side of front, usually as broad as two facets but occasionally only as broad as one. Cuticle nearly always very dark reddish brown to black. Male without a sub-basal pit and associated brush of hairs on ventral side of front femora; male genitalia (figs. 18–19) with parameres more or less evenly narrowed to apex. Length, 4·5–5·5 mm. Africa, Europe, N. America..*destructor* Uyttenboogaart (1933)

13. Pronotum with punctures very much coarser and denser on sides than on disk, being contiguous to confluent on sides and often separated by two diameters on disk. Abdomen with punctures of sides of sternites distinctly coarser and denser than those on disk..14

— Pronotum (fig. 26) with punctures very evenly distributed, being contiguous to confluent on sides and rarely separated by one diameter on disk. Abdomen with punctures of sternites usually as coarse and dense on disk as on sides...15

14. Eyes separated beneath head by the transverse or greatest diameter of the ventral part of an eye. Base of produced tooth-like antero-ventral margin of head opposite base of maxilla contiguous with eye. Elytra with basal three-fourths of sutural interval distinctly carinate. Length, 4·0–4·6 mm. Timbuktu..*downesi* **sp. n.**

— Eyes separated beneath head by twice the greatest diameter of the ventral part of an eye. Base of produced tooth-like antero-ventral margin of head opposite base of maxilla separated from eye by twice the breadth of an eye facet. Elytra with basal three-fourths of sutural interval flat. Length, 3·8 mm. Timbuktu..*semele* **sp. n.**

15. Eye with narrowest part, where divided by side of front, consisting of only three rows of facets; eyes separated beneath head by more than the transverse diameter of the ventral part of an eye (10·5 : 8). Pronotum broad, breadth/length ratio 1·37–1·40; surface without trace of tubercles. Elytra without tubercles on carinae. Length, 3·7–4·5 mm. Abyssinia............*sulmo* **sp. n.**

— Eye with narrowest part, where divided by side of front, consisting of at least four rows of facets; eyes separated beneath head by no more than the transverse diameter of the ventral part of an eye. Pronotum relatively narrower, breadth, length ratio 1·15–1·20; surface with small but usually very distinct, widely spaced tubercles. Elytra with a few irregularly spaced tubercles on discal carinae..16

16. Eyes separated beneath head by once or very little less than once (7 : 7·5) the greatest diameter of the ventral part of an eye; space between eyes considerably broader than postmentum. Elytra with ninth interval distinctly and more or less completely keeled. Pronotum with breadth/length ratio 1·15–1·17. Length, 3·4–4·0 mm. Senegal, Sudan, India...*indicum* Blair (1930)

— Eyes separated beneath head by half (5 : 10) or very little less than half (6 : 10·5) of greatest diameter of ventral part of an eye; space between eyes hardly broader than postmentum (6 : 5·3). Elytra with ninth interval not keeled or only indistinctly so at base. Pronotum with breadth/length ratio 1·20. Length, 3·5–4·0 mm. Chad River, Natal, Cape Province...........*thusa* **sp. n.**

17. Antenna with a distinct 5-segmented club or gradually broadened to apex; eighth segment nearly or quite as broad as ninth. (*alcine* species-group)......18

— Antenna with a sharply differentiated 3-segmented club; eighth segment much narrower than ninth (first of club). (*castaneum* species-group)........20

18. Eyes slightly but distinctly more prominent than sides of front; narrowest part of eye, where divided by side of front, as broad as five or six facets; eyes separated beneath head by less than the greatest diameter of the ventral part of an eye. Antenna with club very abrupt, the seventh segment (first of club) being at least half again as broad as sixth. Length, 3·1–3·5 mm. Madagascar ....*ceto* **sp. n.**

— Eyes distinctly less prominent than sides of front; narrowest part of eye, where divided by side of front, no broader than four facets; eyes separated beneath head by two to nearly three times the greatest diameter of ventral part of an eye. Antenna with club more or less feebly differentiated or without a club 19

19. Species 3·1–4·2 mm. long. Eye with narrowest part, where divided by side of front, as broad as 3½–4 facets. Antenna with a distinct but feebly differentiated 5-segmented club. Madagascar........................*alcine* **sp. n.**

— Species 5·3 mm. long. Eye with narrowest part, where divided by side of front no broader than two facets. Antenna gradually broadened to apex, without a distinct club. Madagascar..*dolon* **sp. n.**

20. Head, pronotum, and elytra with only sparse microscopic punctures about one-seventh as coarse as facets of eyes; surface highly polished. Elytra without striae or carinae but sparsely clothed with long, erect hairs. (Eyes separated beneath head by the transverse diameter of the ventral part of an eye.) Length, 4·5 mm. Doerian Is..*politum* **sp. n.**

— Head, pronotum, and elytra with numerous punctures not less than half as coarse as facets of eyes. Elytra with distinct striae or carinae or both and with short, erect hairs or with hairs so fine as to be scarcely visible..............21

21. Eyes separated beneath head by two to three times the transverse diameter of the ventral part of an eye. Elytra with intervals four to eight distinctly carinate. Length, 3·6–5·5 mm..22

— Eyes separated beneath head by once or very little more than once the transverse diameter of the ventral part of an eye. Elytra distinctly carinate or without distinct carinae. Length, 2·3–4·7 mm..23

22. Eye with narrowest part, where divided by side of front, as broad as four or five facets; eyes separated beneath head by two-and-a-third to three times the transverse diameter of ventral part of an eye. Cuticle very dark brown or black with antennae and legs reddish. Length, 3·6–5·2 mm. Nearly cosmopolitan..*madens* (Charpentier, 1825)

— Eye with narrowest part, where divided by side of front, only as broad as two facets; eyes separated beneath head by twice the transverse diameter of ventral part of an eye. Cuticle entirely reddish brown. Length, 5·5 mm. Kashmir..*freemani* **sp. n.**

23. Surface between punctures of head and pronotum dull or feebly shining and with a dense reticulate microsculpture. Elytra with one or more of intervals four to eight strongly carinate from base to apex except in very rare individuals which have all the intervals flat or nearly so. (Cuticle brownish black to black in *T. castaneum evertsi* Everts, the genetics of which have been briefly described by Miller, 1944, *J. Dep. Agric. Victoria*, **42,** pp. 469–471). Length, 2·3–4·4 mm. Cosmopolitan.....................*castaneum* (Herbst, 1797)

— Surface between punctures of head and pronotum smooth and strongly shining, without a visible microsculpture (at ×75 magnification). Elytra not or at most only indistinctly carinate..24

24. Cuticle black or nearly so. Head with punctures of front seldom separated by as much as one diameter, often confluent. Pronotum with anterior angles, when seen from above, not projecting forwards beyond middle of anterior margin. Prothorax nearly quadrate, breadth/length ratio, 1·03. Length, 3·1–3·5 mm. Malay Peninsula, Batchian.................... *cylindricum* **sp. n.**

— Cuticle moderately dark reddish brown. Head with punctures of front usually separated by one to two or more diameters. Pronotum with anterior angles projecting forwards considerably beyond level of middle of anterior margin. Prothorax distinctly broader than long, breadth/length ratio 1·13–1·34. Length, 3·9–4·7 mm...25

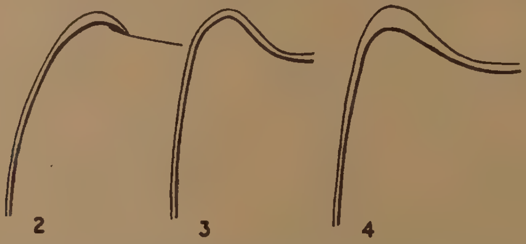
25. Head with punctures of front irregular in shape, about half as coarse as facets of eyes, and mostly separated by two to four diameters. Pronotum slightly but abruptly and distinctly constricted at basal fifth. Antero-ventral margin of head opposite base of maxilla produced to form a stout tooth as long as third and fourth antennal segments together. Discal punctures of first abdominal sternite twice as coarse and dense as those of fourth. Length, 4·7 mm. New South Wales, Queensland...................................... *waterhousei* **sp. n.**

— Head with punctures of front round or nearly so, two-thirds to three-fourths as coarse as facets of eyes, and mostly separated by one or slightly less than one diameter but occasionally by two diameters. Pronotum evenly narrowed to base, without a slight constriction at about basal fifth. Antero-ventral margin of head opposite base of maxilla only slightly produced, the produced part being no longer than third antennal segment. Discal punctures of first abdominal sternite no coarser or denser than those of fourth. Length, 3·9 mm. Larat Island.. *parki* **sp. n.**

Descriptions of new Species.

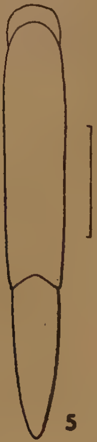
Tribolium carinatum sp. n. (fig. 5).

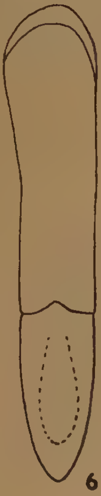
MALE: Length, 4·3–5·3 mm.; breadth, 1·56–1·92 mm. Body subparallel-sided and moderately convex. Cuticle moderately dark reddish brown and feebly shining. Dorsal surface with setae arising from punctures scarcely visible at a magnification of ×75. *Head* with punctures of middle between eyes round or nearly so, two-thirds to four-fifths as coarse as facets of eyes, each with a minute central puncture, and separated mostly by half of one to nearly one diameter; surface between punctures with a dense reticulate microsculpture. Clypeus with anterior margin truncate or nearly so; surface with punctures distinctly finer than those of middle of head, less well impressed, mostly separated by one or little more than one diameter, and surface between punctures slightly more strongly shining. Eye with narrowest part,



Figs. 2–4.—Dorsal view of antero-lateral margin of pronotum. (2) *Tribolium destructor* Uyttenb. (3) *T. brevicornis* (Lec.). (4) *T. parallelus* (Casey).

where divided by side of front, consisting of a single facet (3 specimens) or completely divided (1 specimen) ; eyes separated beneath head by 3–3¼ times the greatest diameter of the ventral part of an eye, the space separating the eyes being approximately three times as broad as postmentum. Antenna with a feebly differentiated 5-segmented club, the seventh segment (first of club) being considerably broader than the sixth but also distinctly narrower than the eighth. *Pronotum* at broadest point, which is usually at about anterior two-fifths, broader than long (1·42 : 1·09 mm.) the breadth/length ratio of the four available specimens varying between 1·29 and 1·31 ; base broader than apex (1·31 : 1·06 mm.). Sides distinctly arcuate, broadly and scarcely noticeably sinuate at middle and again before base ; apical angles, when seen from above, projecting considerably beyond apex, which is distinctly margined on about lateral third, this marginal line being continuous on either side with the lateral marginal line ; base completely and distinctly margined. Disk with punctures round or nearly round, ocellate like those of head, about as coarse as facets of eyes, and separated by half of one to one or, rarely, more than one diameter ; sides with punctures slightly but distinctly coarser, often broadly oval, and rarely separated by as much as one diameter ; surface between punctures with a reticulate microsculpture more lightly impressed on disk than at sides so that sides are less shining than disk. *Elytra* with intervals two to nine distinctly keeled from base to apex, and first (sutural) usually only indistinctly keeled on middle of disk ; accessory sutural interval keeled in all four specimens. Surface of discal intervals with a few widely spaced punctures near or contiguous to keels, these punctures being less distinct but only slightly finer than strial punctures ; surface everywhere with a dense reticulate microsculpture and with a feeble but distinct oily lustre. Strial punctures of disk round, about two-thirds as coarse as those of pronotal disk, and separated longitudinally by two to three or more diameters. *Prosternum* and hypomera with punctures deep, round or oval, as coarse as facets of eyes to slightly coarser, and





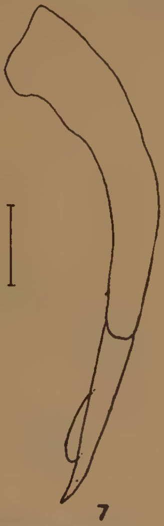


Fig. 5.—*Tribolium carinatum* sp. n. Dorsal view of male genitalia. The lines next to these and other figures refer to a length of 0·20 mm.

Figs. 6–7.—*Tribolium brevicornis* (Lec.). Male genitalia. (6) Dorsal view. (7) Lateral view of left side.

separated by half of one to one diameter, but much sparser on outer part of hypomera; surface between punctures with the usual reticulate microsculpture, which is particularly evident on hypomera. Metasternum with punctures of sides about as coarse as those of prosternum and separated by ½–1½ diameters but considerably finer on both basal and apical parts of sides; punctures of disk only slightly finer and sparser than those of sides. Abdomen with punctures of sides similar to those of metasternal sides but decreasing in size towards apex, so that on fifth sternite they are only two-thirds as coarse as those of first; discal punctures of each sternite slightly finer than those of sides; fifth sternite with marginal line deep, distinct, extending nearly to base, and on apex nearly contiguous to apical margin. Legs with middle femora slightly narrowed near base and ventral side of narrowed part with a number of long hairs; hind femora with basal three-fifths of ventral side shallowly, arcuately emarginate and with numerous long, erect testaceous hairs.

FEMALE: Externally similar to male but with hind femora not emarginate and without long erect hairs on ventral base of middle femora.

TYPE: ♂, in the British Museum (Nat. Hist.). Argentina: La Rioja Prov., Patquia, xii-i. 1932–33 (*K. J. Hayward*).

PARATYPES: 2 ♂♂ and 1 ♀ with same data as type.

COMPARATIVE NOTES: The chief differences between this species and *T. brevicornis* (Lec.) have been listed in the key. From *T. linsleyi* sp. n. it may be distinguished as follows: (1) smaller, 4·3–5·5 : 5·0–6·2 mm. long; (2) eye with narrowest part consisting of a single facet or completely divided; (3) eyes separated beneath head by three or more instead of only two times the greatest diameter of the ventral part of an eye; (4) fifth abdominal sternite with marginal groove nearly contiguous to apical margin instead of being separated from apical margin by a distance equal to about twice the diameter of an eye facet; and (5) the cuticle of the dorsal surface is much less shining and has a distinct oily lustre not evident in the available specimen of *T. linsleyi* sp. n.

Tribolium gebieni Uyttenb. is probably very closely related to *T. carinatum*, but may be distinguished by its much more coarsely facetted eyes. Uyttenboogaart claims that the eyes of *T. gebieni* are much more coarsely facetted than those of *T. destructor*, whereas in *T. carinatum* the facets of the eyes are no coarser than those of *T. destructor*.

Tribolium carinatum dubium subsp. n.

MALE: Length, 4·3–5·1 mm.; breadth, 1·39–1·77 mm. Externally similar to *T. carinatum* but with the eyes less completely divided, the narrowest part of the eye where divided by the side of the front containing two to three facets instead of not more than one or being completely divided as in *T. carinatum*. The secondary sexual characters of the male are exactly like those of *T. c. carinatum*, nor does there appear to be any difference between the two as regards the structure of the male genitalia. More specimens, particularly from intermediate regions, may reveal a continuous series as regards the division of the eyes.

TYPE: ♂, in the collection of the Museum of Natural History, Paris. Argentina: Chaco de Santiago del Estero, Rio Salado, near Icano, 1910 (*E. R. Wagner*).

PARATYPES: 2 ♀♀, Chaco de Santiago del Estero, La Palisa del Bracho, 25 km. from Icano at Laguna Mamaita, 1904 (*E. R. Wagner*).

Tribolium linsleyi sp. n. (fig. 8).

1893. *Aphanotus parallelus* Casey, Champion, Biol. Centr.-Amer., Col. 4 (1) p. 534.

FEMALE: Length, 6·5 mm.; breadth, 2·1 mm. Cuticle moderately dark reddish brown and moderately strongly shining, without an evident oily lustre. Body similar in shape to *T. parallelus* (Casey). Its chief distinguishing features are as follows: *Head* with punctures of front round or nearly so, distinctly ocellate, about two-thirds as coarse as facets of eyes, and separated by half of one to nearly one diameter; surface between punctures with a very lightly impressed microsculpture. Clypeus with anterior margin nearly truncate; surface distinctly more finely and sparsely punctate than head. Eyes large, dorsal part as long as head in front of eyes; narrowest part, where divided by side of front, as broad as three facets; eyes separated beneath head by twice the diameter of the ventral part of an eye. Antenna with a 5-segmented club. *Pronotum* with breadth/length ratio 1·14; apex, except middle third, distinctly margined; apical angles, when seen from above, less than twice as thickly margined as sides. Disk with punctures slightly coarser, deeper, and less distinctly ocellate than those of head and mostly separated by one to two diameters; on sides punctures are as coarse as, to slightly coarser than, facets of eyes and are oval and denser than those of disk; surface between punctures strongly shining and smooth or with a scarcely noticeably impressed microsculpture. *Elytra* with intervals two to eight distinctly carinate from base to near apex; ninth interval less distinctly carinate from near base to apical fourth; accessory sutural interval distinctly carinate; first (sutural) interval distinctly margined from apex to near scutellum. *Abdomen* with marginal line of fifth sternite complete nearly to base and on middle of apex separated from apex by nearly twice the diameter of an eye facet.

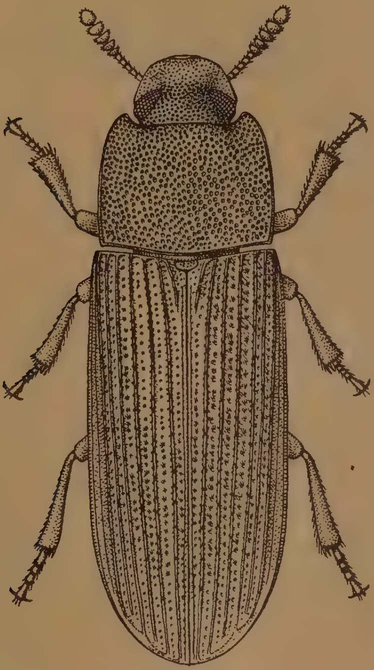


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Fig. 8.—*Tribolium linsleyi* sp. n.

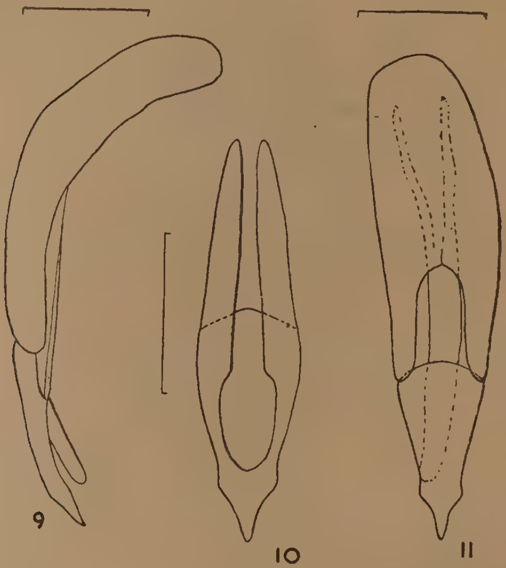
MALE : Unknown.

TYPE : ♀, in the British Museum (Nat. Hist.). Mexico : Colima, Zapotlan (*Höge*).

COMPARATIVE NOTES : From *T. parallelus* (Casey) it differs not only in having the anterior angles of the pronotum much less thickly margined but also in having the eyes completely divided. Casey (1890, Ann. N.Y. Acad. Sci., **5,** p. 483) and Blaisdell (1942, Trans. Amer. ent. Soc., **68,** p. 146) both claim that the eyes of *T. parallelus* are not completely divided, but in all the specimens I have seen from several localities in Arizona they are completely divided. This is true also of three specimens from the St. Xavier Mts. which were examined by Blaisdell himself and which were kindly sent to me by Dr. E. S. Ross.

Tribolium anaphe sp. n. (figs. 9–11).

MALE : Length, 3·5–5·08 mm. ; breadth, 1·12–1·64 mm. Externally similar to *T. confusum* from which it may be distinguished as follows : (1) length, 3·59–5·08 mm. with an average of 4·35 mm., whereas *T. confusum* varies from 2·62 mm. to 4·39 mm. with an average of 3·95 mm.[1] ; (2) eye with narrowest part, where divided by side of front, consisting of two or three instead of only one facet ; (3) eyes separated beneath head by $1\frac{2}{5}$ to $2\frac{1}{3}$ instead of $2\frac{1}{2}$ to $3\frac{1}{5}$ times the diameter of the ventral part of an eye, the ratio space/eye being 1·40–2·33 instead of 2·50–3·20 ; (4) middle posterior margin of ventral part of eye not raised and distinctly below level of most convex



Figs. 9–11.—*Tribolium anaphe* sp. n. (9) Lateral view of right side of male genitalia. (10) Ventral view of parameres. (11) Dorsal view of genitalia.

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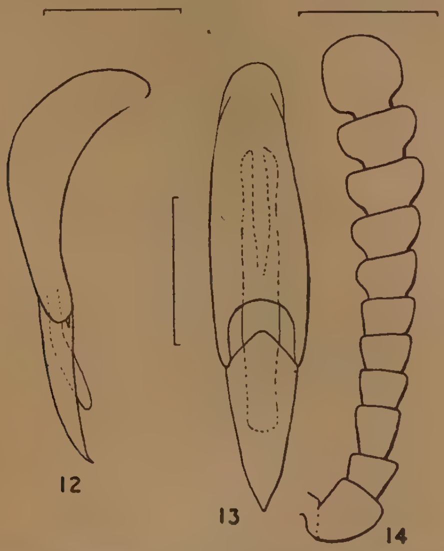
[1] Good (1936), *Tech. Bull. U.S. Dep. Agric.* **498,** gives the length of *T. confusum* as 3·09 to 3·82 mm., with an average of 3·45 mm. If his measurements were taken correctly, his specimens must have been taken from a small strain or from a culture bred at fairly high temperature. The average length of the specimens from many different parts of the world in the British Museum is greater than the maximum length given by Good. Perhaps of greater significance than the average is the mode, which in my specimens is between 4·05 mm. and 4·11 mm.

part of eye, whereas in *T. confusum* it is slightly raised in most specimens so that it is nearly as high as most convex middle ventral part of eye ; and (5) the male genitalia have the fused lateral lobes abruptly constricted before apex (figs. 10–11) instead of evenly narrowed to apex (fig. 13).



Figs. 12–14.—*Tribolium confusum* J. du V. (12) Lateral view of right side of male genitalia. (13) Dorsal view of same. (14) Antenna.

Female : Externally similar to male but without the sub-basal pit and associated brush of hairs present in the male on the ventral side of the front femur.

Type : ♂, in the British Museum (Nat. Hist.). N. Nigeria : Yola Province, viii.1920, *ex* cocoons of *Anaphe moloneyi* Druce.

Paratypes : 4, with same data as type ; 1, Chari-Chad, Fort Sibut ; 1, Upper Senegal, Koulikoro (*Keisser*) ; 1, Uganda, " found on dried Lepidoptera from Uganda " (*G. A. K. Marshall*) ; 1, Belgian Congo : 18 miles S.W. of Elizabethville, 31.iii.1928 (*H. S. Evans*) ; 2, Mashonaland : Salisbury, iv.1899, under dead bark (*G. A. K. Marshall*).

Comparative Notes : In addition to the differences listed above, the elytra of *T. anaphe* are usually more strongly carinate than in *T. confusum*, and the ninth interval is more or less distinctly keeled in most specimens, whereas this interval is not keeled in any specimens of *T. confusum* examined. Some of the smaller individuals of *T. anaphe* have the ninth interval quite flat and the eighth only feebly keeled. The male of *T. anaphe* lacks the brush of hairs present in *T. confusum* near the ventral base of the middle and hind femora.

T. anaphe is also very closely related to *T. destructor*, from which it may be distinguished as follows : (1) the cuticle is moderately dark reddish brown as in *T. confusum* instead of very dark reddish brown to black, as is nearly always the case in *T. destructor* ; (2) the punctures of the head are relatively smaller and usually round instead of obovate, and very few or none of the punctures are longitudinally confluent ; (3) the narrowest part of the eye is as broad as two or three instead of only one or two facets ; (4) the male has a distinct sub-basal pit and brush of hairs on the ventral side of the front femora, whereas the pit and distinct brush of hairs are absent in the

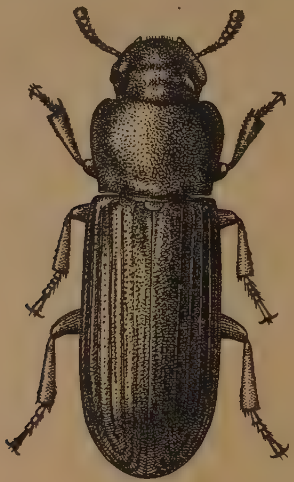


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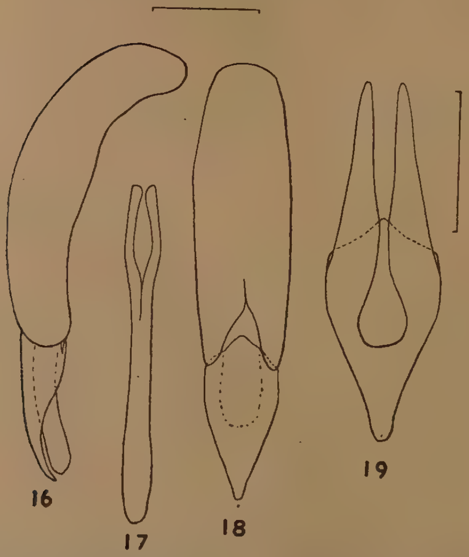
Fig. 15.—*Tribolium confusum* J. du V.



Figs. 16–19.—*Tribolium destructor* Uyttenb. (16) Lateral view of right side of male genitalia. (17) Dorsal view of median lobe. (18) Dorsal view of genitalia. (19) Ventral view of parameres.

male of *T. destructor*, although the homologous area in the latter species is more densely punctate in the male than in the female ; and (5) the parameres (fig. 10) are abruptly constricted before the apex instead of rather evenly narrowed to the apex, as in *T. destructor* (fig. 19). *T. destructor* is much less variable in size than *T. anaphe*, nearly all the specimens seen being within the range 5·0–5·5 mm. long, most of them being slightly larger than my largest specimen of *T. anaphe.*

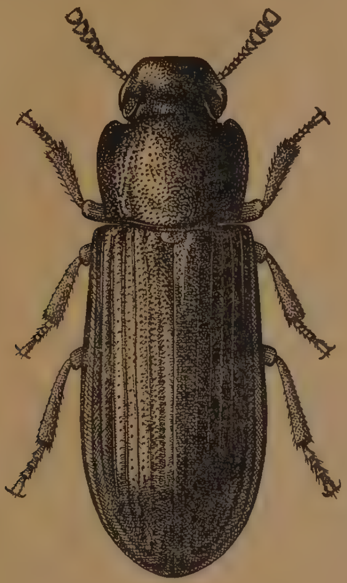


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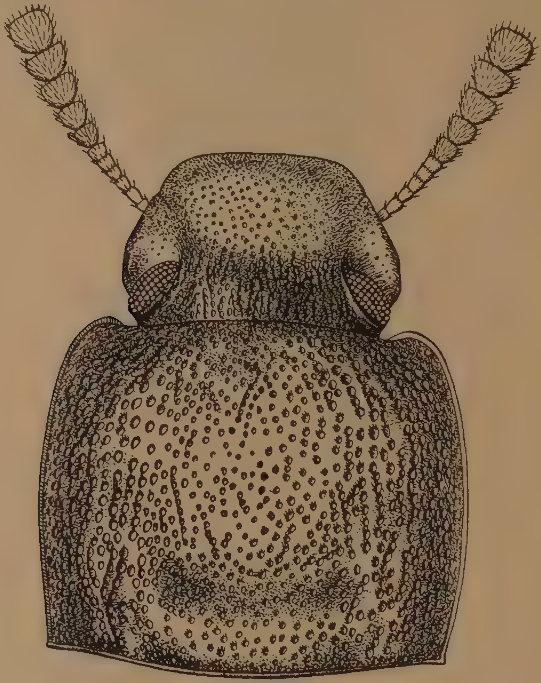
Fig. 20.—*Tribolium destructor* Uyttenb.

I am indebted to Dr. T. H. C. Taylor for the information that a species of *Tribolium*, presumably *T. anaphe*, is very common in the nests of various species of *Anaphe* in Uganda, where many specimens may be found in a single nest.

Tribolium giganteum sp. n. (figs. 22–24).

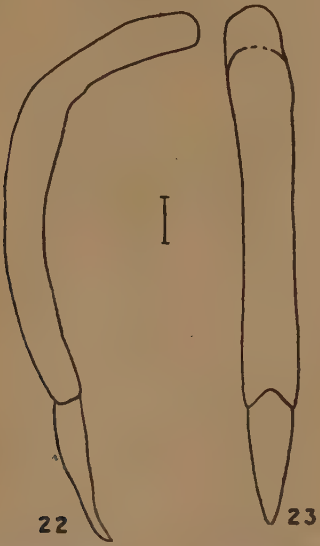
MALE : Length, 10–12 mm. ; breadth, 3·7–4·5 mm. Body subparallel-sided and moderately convex with disk of elytra nearly flat. Cuticle strongly shining and black or nearly so with antennae and legs very dark rufo-piceous to black. Setae of dorsal surface inconspicuous, about as long as the punctures from which they arise. *Head* with punctures of middle of front round, moderately deep, slightly but distinctly coarser than facets of eyes, and usually separated by less than one to one diameter but on some areas separated by two or even three diameters ; surface between punctures with an inconspicuous and lightly impressed reticulate microsculpture ; margins above eyes strongly flexed upwards and forming a very conspicuous ridge. Clypeus with anterior margin very broadly, moderately deeply, truncately emarginate ; fronto-clypeal suture distinct at sides and indicated across middle by a moderate depression ; surface with punctures only slightly finer than those of head and usually separated by 1–1½ diameters, but denser in some specimens. Eye with narrowest part, where divided by side of front, consisting of a single facet ; eyes separated beneath head by very slightly more or very slightly less than twice the transverse or greatest diameter of the ventral part of an eye, the space between the eyes being twice as broad as the postmentum. Antenna with club 7-segmented, the fifth segment (first of club) being considerably broader than fourth but also narrower than sixth; third segment twice as long as second (8 : 4) and at apex slightly broader than second. *Pronotum* at broadest point, which is at about apical two-fifths, broader than long (4·11 : 2·79 mm.), the breadth/length ratio varying from 1·3 to 1·4; base broader than apex (3·83 : 2·56 mm.). Sides arcuate, particularly in apical half, and thickly, completely margined, the area immediately lateral from the outer vertical side being wide, flat, and feebly inclined; apical angles, when seen from above, distinctly projecting beyond apex which is unmargined and broadly, shallowly, arcuately emarginate at middle; base completely margined, the marginal line being distinctly thicker at middle than at sides. Pronotum with a broad moderately deep impression on each side before base and with a broad transverse impression on middle of base. Middle of disk with round punctures slightly finer than facets of eyes and somewhat irregularly distributed, being separated by one to three diameters; sides with oval punctures which are usually as coarse as, to slightly coarser than, facets of eyes and are separated by half of one to one diameter or, occasionally, two diameters; surface between punctures smooth or with a very lightly impressed, scarcely noticeable (mag. ×75) reticulate microsculpture. *Elytra* with sixth interval strongly carinate from near base to about



21

Fig. 21.—*Tribolium destructor* Uyttenb.

apical fourth, and fifth interval similarly but less distinctly carinate, the area between the two carinae being moderately strongly concave on basal region ; intervals three, four, and eight feebly convex. Discal area sculptured like pronotal disk but with reticulate microsculpture between punctures slightly more evident ; strial punctures not distinct from those of intervals ; apex of elytra very densely and more coarsely punctate than disk. *Prosternum* nearly impunctate on middle but at sides with punctures finer than facets of eyes and separated by one to three diameters except near anterior margin, where they are confluent. Hypomera punctate like sides of prosternum but more sparsely so. Metasternum with disk only very finely and sparsely punctate and sides with round punctures about as coarse as facets of eyes and separated by one to three diameters. Abdominal sternites one to four with discal region finely and sparsely punctate and sides with punctures two to three times as coarse and much denser ; fifth sternite with coarse and dense punctures both on disk and sides. All sternites completely margined except fifth, which is margined nearly to base ; marginal line of fifth 1–1½ facets from margin ; third and fourth sternites with lateral fourth of base also margined ; disk of second and third or, in some specimens, first three sternites feebly depressed.



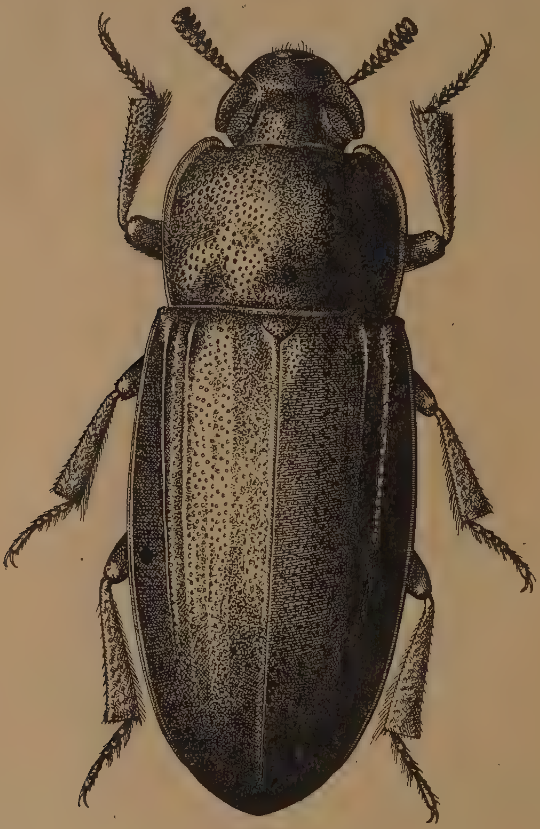
Figs. 22–23.—*Tribolium giganteum* sp. n. Male genitalia. (22) Lateral view of right side. (23) Dorsal view.

FEMALE : Externally similar to male but without a sub-basal pit and brush of hairs on ventral side of front and middle femora and without a sub-basal tubercle on ventral side of hind femur.

TYPE : ♂, in the Museum of Natural History, Paris. Kenya : Voi River, 1904 (*C. Alluaud*).

PARATYPES : 5 ♀♀ and 8 ♂♂ with same data as type.

COMPARATIVE NOTES : This species is not only very much larger than any other member of the genus but is unique in having the antennal club 7-segmented and in having the fifth and sixth intervals conspicuously carinate, particularly near the base. Of the known species, it is nearest to *T. confusum*, with which it agrees in having in the male the sub-basal pit and associated brush of hairs on the ventral side of the front and middle femora, but differs in having a small but conspicuous tubercle on the ventral side of the hind femur. The male of *T. confusum* has a few closely placed hairs, usually not more than 2–4, on the ventral side of the hind femur, but the hairs do not arise from a distinct pit as do those of the front femur.



24

Fig. 24.—*Tribolium giganteum* sp. n.

Tribolium downesi sp. n.

Male : Length, 4·0–4·6 mm. ; breadth, 0·95–1·18 mm. Body subparallel-sided, moderately convex. Cuticle dark reddish brown to nearly black and moderately feebly shining. Dorsal surface with setae scarcely visible at a magnification of ×75. *Head* with punctures of middle between eyes oval to nearly round, shallow, about two-thirds as coarse as facets of eyes, and usually separated by about one diameter ; surface between punctures with a distinct reticulate microsculpture ; margin above each eye flexed upwards to form a low but distinct ridge. Clypeus with anterior margin, when seen from front, truncate or nearly so ; surface with punctures only half as coarse as those of front of head and much sparser. Eye with narrowest point, where divided by side of front, as broad as 4½–5 facets; eyes separated beneath head by the transverse or greatest diameter of the ventral part of an eye (9 : 9), the space between the eyes being considerably broader than postmentum (10 : 6). Antenna with club abruptly 5-segmented, the seventh segment (first of club) being approximately as broad as eighth and very much broader than sixth. *Pronotum* at broadest point, which is at about apical two-fifths, slightly broader than long (1·01 : 0·93 mm.) and base broader than apex (0·93 : 0·74 mm.). Sides distinctly arcuate and distinctly and broadly sinuate before hind angles, which are prominent; apical angles, when seen from above, not projecting as far forwards as middle of apex, which is unmargined; base completely margined, the margin being slightly thicker at middle than at sides. Disk with punctures of middle region slightly finer than those of head, distinctly finer than facets of eyes, round to broadly oval, and usually separated by one to two diameters but occasionally by only half a diameter; towards sides punctures become denser and coarser so that on lateral third or fourth they are 1⅛–1⅔ as long as facets of eyes and are usually separated by only one-third of a diameter; surface between punctures everywhere with a very strongly impressed reticulate microsculpture. *Elytra* with first eight intervals distinctly keeled from base to apex and ninth interval scarcely noticeably to feebly keeled on basal region. Surface of discal region with reticulate microsculpture less distinct than that of pronotum and with a few small, indistinct punctures near carinae. Discal strial punctures round, about half as coarse as facets of eyes, and usually separated by two to three diameters. *Prosternum* with punctures moderately dense and ½–⅔ as coarse as facets of eyes. Hypomera coarsely, rugosely punctate and with a few distinct oblong tubercles. Metasternum with punctures of sides oval, two-thirds as coarse as facets of eyes, and separated by half of one to one diameter; disk with round punctures considerably finer and sparser than those of sides. Abdominal sternites with punctures of sides distinctly coarser than those of disk; fifth sternite with marginal line nearly contiguous with apical margin, sub-dorsal, and rather indistinct in most specimens; each side of fifth sternite with a nearly round, moderately deep, basal depression.

FEMALE: Externally similar to male.

TYPE: ♂, in the Museum of Natural History, Paris. French Sudan: Timbuktu, 1911 (*F. de Zeltner*).

PARATYPES: 6, with same data.

COMPARATIVE NOTES: This and the species described immediately below are the only members of the *downesi* section of the *confusum* species-group which have the punctures of the sides of the pronotum very much coarser and denser than those of the middle. It differs from the closely related *T. semele* sp. n. as shown under the heading of the latter. I take pleasure in naming this species after Mr. J. A. Downes.

Tribolium semele sp. n.

MALE: Length, 3·8 mm.; breadth, 1·01 mm. Externally similar to *T. downesi* except as follows: (1) eyes much smaller, being separated beneath the head by twice the greatest diameter of the ventral part of an eye; (2) the base of the produced, tooth-like, antero-ventral margin of the head opposite the base of the maxilla is separated from the eye by twice the breadth of an eye facet, whereas in *T. downesi* the base of the produced tooth-like margin is contiguous with the eye; and (3) the basal three-fourths of the sutural interval is flat instead of being distinctly carinate.

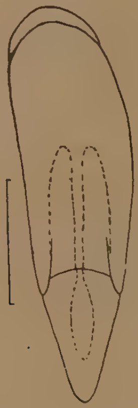
FEMALE: Externally similar to male.

TYPE: ♂, in the Museum of Natural History, Paris. French Sudan: Timbuktu, 1911, (*F. de Zeltner*).

PARATYPE: ♀, with same data in the British Museum (Nat. Hist.).

Tribolium sulmo sp. n. (fig. 25).

MALE : Length, 3·7–4·5 mm. ; breadth, 1·37–1·67 mm. Body subparallel-sided and moderately convex with disk of elytra nearly flat. Cuticle only feebly shining and dark reddish brown. Dorsal surface only microscopically setose, the setae being difficult to see at ×75. *Head* with punctures shallow but distinct, round or nearly so, about two-thirds as coarse as facets of eyes, and mostly separated by half of one diameter ; surface between punctures with a dense reticulate microsculpture so that it is nearly mat ; margins above eyes flexed upwards and ridge-like. Clypeus with anterior margin truncate or nearly so ; surface sculptured like middle of head but with punctures slightly finer and less distinct. Eye with narrowest part, where divided by side of front, as broad as three facets ; eyes separated beneath head by more than the transverse diameter of ventral part of an eye (10·5 : 8 ; 12 : 8), the space between the eyes being twice as broad as postmentum (12 : 6). Antenna with club abruptly 5-segmented, the seventh segment (first of club) being very nearly as broad as eighth and very much broader than sixth. *Pronotum* at broadest point, which is at about middle, broader than long (1·39 : 1·01 mm.) and base broader than apex (1·23 : 0·95 mm.). Sides strongly arcuate and broadly and distinctly sinuate before basal angles, which are prominent ; apical angles, when seen from above, slightly but distinctly projecting beyond apex, which is arcuate and unmargined ; base completely margined. Disk with punctures round or nearly so, deeper but scarcely coarser than those of middle of head, and separated by half of one to one diameter with many punctures on mid-discal region separated by their diameters ; all of sides with punctures as coarse as facets of eyes to slightly but distinctly coarser, round or irregular in shape, and contiguous but rarely confluent, always separated by much less than one diameter ; surface between punctures everywhere with a dense, well impressed, reticulate microsculpture so that the surface is only feebly shining. *Elytra* with intervals two to nine strongly carinate from base to apex ; sutural interval feebly carinate or flat on disk but distinctly carinate on apical third and near base. Discal strial punctures about two-thirds as coarse as those of mid-discal region of pronotum, arranged in rather irregular rows, and longitudinally separated mostly by two to four diameters ; intervals with indistinct punctures similar to strial punctures near the tops of the carinae, everywhere densely and microscopically



25

Fig. 25.—*Tribolium sulmo* sp. n. Dorsal view of male genitalia.

reticulate, and with numerous shallow, transverse or oblique rugae. *Prosternum* and hypomera feebly, indistinctly rugose and with punctures two-thirds to three-fourths as coarse as facets of eyes and separated by one to two diameters, but sparser on outer part of hypomera. Metasternal sides punctate like disk of pronotum but slightly more sparsely so; disk with punctures one-third to one-half as coarse as facets of eyes and usually separated by two to three diameters. Abdominal sternites punctate like pronotal disk but slightly more sparsely so, the lateral punctures being similar to the discal and both discal and lateral punctures scarcely smaller on the fourth and fifth sternites; fifth sternite with a very fine and indistinct apical marginal line.

FEMALE: Externally similar to male.

TYPE: ♂, in the British Museum (Nat. Hist.). Abyssinia (*Raffray*).

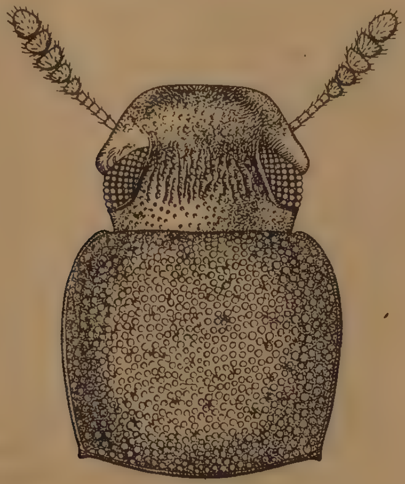
PARATYPE: ♀, with same data as type.

COMPARATIVE NOTES: This is closely related to *T. indicum* Blair, but may be distinguished as follows: (1) the narrowest part of the eye is as broad as three instead of 4–4½ facets; (2) the eyes are separated beneath the head by slightly more than the transverse (greatest) diameter of the ventral part of the eye; (3) the breadth/length ratio of the pronotum is 1·37–1·40 instead of 1·15–1·17; and (4) there are no tubercles on the pronotum as in *T. indicum* (fig. 26).

Tribolium indicum Blair (fig. 26).

1930. *Tribolium indicum* Blair, Indian Forest Rec., **14,** p. 16.

The type and another specimen from the same locality in the Punjab (Chichawa, Montgomery Division) have been examined. The species is very close indeed to *T. sulmo* sp. n. from Abyssinia and *T. thusa* sp. n. from various parts of Africa, and may, moreover, prove to be identical to one or both of the new forms described below. It belongs to an Ethiopian species-group. All Indian and other Oriental species belong to the *castaneum* species-group. For these reasons I believe that it is extremely likely that *T. indicum* is an African species recently introduced by man into India.



26

Fig. 26.—*Tribolium indicum* Blair, *ares* f. n.

If this is so, the typical form still remains to be discovered in Africa, or, if the two new forms described below are later shown to be specifically distinct from the two Indian specimens, *T. indicum* has still to be found in Africa.*

The two African specimens differ rather strikingly from each other and from the two Indian specimens of *T. indicum* in the type of punctures of the middle of the head. These differences in the punctures of the head are of greater magnitude than those found in long series of *T. confusum*, *T. castaneum* or other species. On the other hand, the differences between the punctures of the pronotum, and particularly the carinae of the elytra, are much less than are often met with in a small series of *T. confusum* or *T. castaneum*. I am unable to designate the African specimens as subspecies, since the necessary geographical or other evidence is lacking; and I hesitate to describe them on such insufficient material. The forms of *T. indicum* may be distinguished as follows:

1. Punctures of middle of head between eyes obovate and frequently longitudinally confluent so that the surface is distinctly strigose (fig. 26). Marginal line of fifth abdominal sternite fine but very distinct and complete nearly to base. Length, 3·71 mm. Sudan: Khor Arbaat Delta, iv-v. 1926, at light (*H. B. Johnston*) .... *Tribolium indicum ares* **f. n.**

— Punctures of middle of head between eyes round or broadly oval and if confluent not longitudinally so; surface not strigose. Marginal line of fifth abdominal sternite fine and very indistinct.. 2

2. Head between eyes with punctures round or nearly so, as large as facets of eyes, and rarely separated by as much as half of one diameter, frequently confluent. Length, 3·42 mm. Senegal (*Toulze*).............. *Tribolium indicum seres* **f. n.**

— Head with punctures between eyes slightly finer than facets of eyes and usually separated by about half of one diameter, rarely confluent. Length, 3·3–4·01 mm. India (introduced ?).. *Tribolium indicum* Blair (1930) f. typ.

Tribolium thusa sp. n. (fig. 27).

MALE: Length, 3·59–4·00 mm.; breadth, 1·12–1·23 mm. Body subparallel-sided and moderately convex. Cuticle dark reddish brown and moderately feebly shining. Dorsal surface with setae scarcely visible at a magnification of ×75. *Head* with punctures of middle between eyes usually round but occasionally oval, shallow, two-thirds to three-fourths as coarse as facets of eyes, and more or less contiguous though rarely confluent; surface between punctures with a lightly impressed reticulate microsculpture; margins above eyes strongly flexed upwards and each forming a very distinct ridge. Clypeus with anterior margin scarcely noticeably rounded (type) or truncate (paratype); surface with punctures about two-thirds as coarse as those of head, more oval, and often separated by nearly one diameter; surface between punctures with a very lightly impressed microsculpture. Eye with narrowest part, where divided by side of front, as broad as 4½–5 facets; eyes separated beneath head by half or little more than half of one diameter (5 : 10; 6 : 10·5), the space between the eyes being little broader than postmentum (6 : 5·3). Antenna with club very abruptly 5-segmented, the seventh segment (first of club) being very nearly as broad as eighth and very much broader than sixth. *Pronotum* at broadest point, which is at about middle or slightly before, broader than long (1·10 : 0·91 mm.) and base broader than apex (0·93 : 0·82 mm.). Sides distinctly arcuate and scarcely noticeably sinuate before hind angles, which are prominent; apical angles, when seen from above, hardly projecting beyond apex, which is unmargined; base completely and thickly margined. Disk with punctures slightly coarser than those of middle of head or only very slightly finer than facets of eyes, usually round but occasionally oval, deeper than those of head, and mostly separated by about half of one diameter or less, only on apex being separated occasionally by as much as one diameter; at sides punctures are about as coarse as facets of eyes, slightly denser, and each has a minute more or less central puncture more distinct than the central punctures in the coarse discal punctures; surface between punctures with numerous tubercles about half as coarse as large punctures. *Elytra* with intervals two to eight keeled from base to apex, the ridges or keels of second, and to a lesser extent of third and fourth intervals, being broadly interrupted on discal area; second and third intervals not distinctly keeled on discal region in one of the paratypes; first (sutural) interval carinate on apical fourth but elsewhere without a distinct keel but with the numerous widely spaced tubercles (as coarse as strial punctures) which are situated on tops of the keels of other intervals. Surface of discal intervals with a lightly impressed reticulate microsculpture (mag. × 75) and with numerous irregular rugae which are usually transverse or oblique; punctures slightly finer than strial punctures and sparse and indistinct except on first interval where they are separated mostly by one to three diameters. Discal strial punctures round, nearly two-thirds as coarse as those of pronotal disk, arranged in somewhat irregular rows, and separated longitudinally mostly by two to three diameters. *Prosternum* with round or oval punctures two-thirds as coarse as facets of eyes and mostly separated by less than their diameters. Hypomera punctate like prosternum but with punctures slightly shallower and sparser, but near dorsal margin with a broad nearly impunctate belt; surface of dorsal impunctate belt and elsewhere between punctures with reticulate microsculpture much more distinct than that of prosternum. Metasternum with punctures of sides round, about two-thirds as coarse as facets of eyes and separated by less than one to one diameter; disk with punctures about two-thirds as coarse as those of sides and separated by one to three diameters but much denser on

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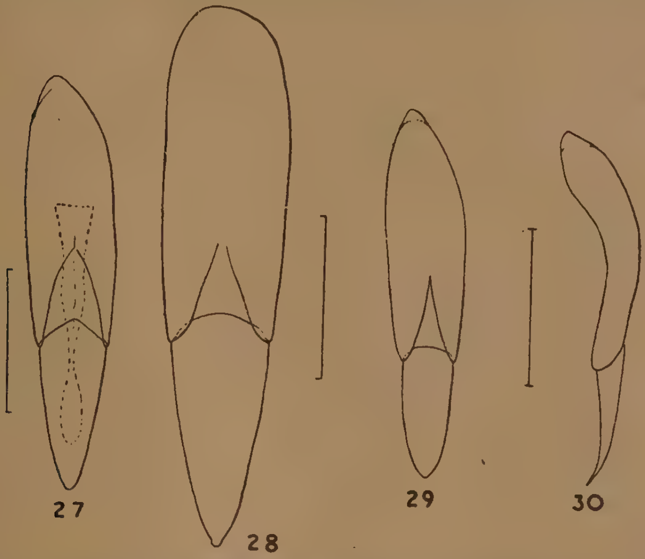
* Since the above was written, I have seen a specimen of *T. indicum* Blair typical form with data as follows: French Somaliland: Obock, 1893 (*Maindron*).



Figs. 27–28.—(27) *Tribolium thusa* sp. n. Dorsal view of male genitalia. (28) *Tribolium dolon* sp. n. Dorsal view of male genitalia. Figs. 29–30.—*Tribolium ceto* sp. n. Male genitalia. (29) Dorsal view. (30) Lateral view of left side.

anterior seventh. Abdomen with punctures of sides of sternites slightly coarser than those of disk and similar to those of metasternal sides; second and third sternites with punctures of disk slightly but distinctly sparser than those of sides; fifth sternite without a marginal line.

FEMALE: Externally similar to male.

TYPE: ♂, in the British Museum (Nat. Hist.). S. Africa: Cape Province, Somerset East, x. 1930 (*R. E. Turner*).

PARATYPES: 1, with same data as above but collected in November; 1, Natal, Estcourt (*G. A. K. Marshall*); and 1, Moyen Chari, Est des Niellims, Komé (Kouom), v. 1904 (*J. Decorse*).

COMPARATIVE NOTES: In general appearance and in the sculpture of the dorsal and ventral surfaces, this species is nearly identical to *T. indicum* Blair (fig. 26). The punctures of the middle of the head are not longitudinally confluent in the available specimens, as is the case in some of the forms of *T. indicum*. The ninth interval of the Indian and African specimens of *T. indicum* is distinctly keeled, whereas in the new species this interval has either no trace of a keel or is only very indistinctly keeled near its base. The striking difference between the two, however, is in the structure of the eyes, as described in the key. *T. thusa* sp. n. has the eyes much closer together beneath the head than any other known *Tribolium*.

Tribolium alcine sp. n.

MALE: Length, 3·1–4·2 mm.; breadth, 0·98–1·31 mm. Body similar in shape and convexity to *T. castaneum*. Cuticle moderately shining and dark reddish brown to nearly black; antennae and legs always slightly paler. Dorsal surface with microscopic setae difficult to see at ×75. *Head* with punctures of middle between eyes usually oval, about two-thirds as coarse as facets of eyes, and usually separated by half of one to one diameter, but in some specimens some punctures are confluent; surface between punctures with a distinct reticulate microsculpture; margins above eyes not ridged. Clypeus with anterior margin, when seen from front, truncate or nearly so; surface with punctures as dense but distinctly finer than those of head; middle of anterior margin with a narrow, nearly impunctate belt. Eye with narrowest part, where divided by side of front, as broad as 3½–4 facets; eyes separated beneath head by slightly more than two to nearly three times the transverse or greatest diameter or ventral part of an eye (12 : 5·5–17 : 6), the space between the eyes being two to three times as broad as postmentum. Antenna with club 5-segmented, the seventh segment (first of club) being slightly but distinctly broader than sixth; third segment very distinctly narrower and shorter than second. *Pronotum* at broadest point, which is just before middle, broader than long (1·23 : 0·98 mm.) and base broader than apex (1·09 : 0·79 mm.). Sides strongly arcuate, hardly noticeably sinuate behind hind angles, and completely margined; apical angles, when seen from above, hardly projecting beyond middle of apex, which is unmargined; base completely and thickly margined. Disk with punctures of middle region similar in size to those of head but rounder and separated in some specimens usually by one diameter but occasionally by as much as three and in other specimens by half of one to one diameter; nearly all specimens with a narrow, indistinct, more or less impunctate median longitudinal line on anterior half of disk; sides with punctures slightly coarser and denser than those of disk; surface between punctures with a dense, well impressed, reticulate microsculpture and with numerous round callosities one-half to two-thirds as coarse as punctures, these callosities being distinct in most specimens at a magnification of ×75. *Elytra* with intervals two to seven distinctly keeled from base to near apex, eighth usually much less distinctly keeled, and ninth flat or only feebly convex, often convex only near base; first or sutural interval with marginal line of suture distinct on apical three-fourths, but in a few specimens distinct to base;

accessory sutural interval feebly convex, sometimes subcarinate. Surface of discal intervals irregularly and shallowly rugose, with an evident reticulate microsculpture, and on either side of each carina with a row of punctures which are slightly finer than strial punctures. Discal strial punctures as fine as those of middle of pronotal disk (distinctly finer in some of the smaller specimens) and usually separated longitudinally by two or three diameters. *Prosternum* with deep, oval punctures one-half to two-thirds as coarse as facets of eyes and separated by one to three diameters, but denser at sides. Hypomera with punctures like prosternal sides but sparser near pronotal margin. Metasternal sides with round or oval punctures nearly as coarse as facets of eyes and separated by one to three diameters ; middle of disk with microscopic punctures separated by four to ten diameters. Abdomen with punctures of first three sternites nearly as dense but only about half as coarse on disk as on sides ; sternite four and five with discal punctures very nearly or quite as coarse as those of sides ; fifth sternite with apical marginal line very close to apex and nearly complete to base.

FEMALE : Externally similar to male but without a sub-basal pit and brush of hairs on ventral side of front and middle femora, and without the inconspicuous sub-basal brush consisting of only a few fine hairs on ventral side of hind femora.

TYPE : ♂, in the Museum of Natural History, Paris. Madagascar : Ambohabe, Mondre, 1891 (*Douliot*).

PARATYPES : 8, with same data as type and 8 also from Madagascar with data as follows : 5, Region de Morondava, 1901 (*Petit*) ; 1, Plaines de Finerena, 1905 (*F. Geay*) ; 1, near Marovoay, 1911 (*J. Descarpentries*) ; and 1, without further data.

COMPARATIVE NOTES : From all African species it may be distinguished by not having the margins above the eyes ridged, and from all Indo-Australian species by having a 5-segmented antennal club together with a completely margined prothoracic base. It is the only member of its species-group that has the sub-basal pits and associated brush of hairs on the femora of the male. It is the most primitive of the Madagascan species as is shown by its secondary sexual characters and feebly differentiated 5-segmented antennal club.

Tribolium dolon sp. n. (fig. 28).

MALE : Length, 5·3 mm. ; breadth, 1·7 mm. Similar in general appearance to *T. alcine*, sp. n. Its chief structural features are as follows : *Head* with distinctly oval (often twice as long as broad) punctures which are about as long as facets of eyes and are usually separated by half of one to one length ; surface between punctures with a scarcely noticeable reticulate microsculpture and with a distinct oily lustre ; margins above eyes not ridged though from some angles they appear to be very slightly ridged. Clypeus with anterior margin, when seen from front, very broadly and shallowly, arcuately emarginate ; surface with punctures much finer, rounder, and considerably sparser than those of head. Eye with narrowest part, where divided by side of front, only as broad as two facets ; eyes separated beneath head by twice (20 : 10) the transverse or greatest diameter of the ventral part of an eye, the space between the eyes being slightly more than twice as broad as postmentum. Antenna with a scarcely distinguishable 5-segmented club, the segments being gradually broadened towards apex as, for instance, in *Palorus* ; apical segment slightly but distinctly narrower than tenth ; third segment slightly narrower and no longer than second. *Pronotum* with breadth/length ratio 1·24. Sides arcuate and narrowly sinuate before hind angles, which are somewhat produced and prominent ; sides and base completely margined ; apex unmargined and apical angles, when seen from above, projecting slightly but distinctly beyond middle of apex. Disk with punctures of middle slightly but distinctly finer than those of head, round or nearly round, and usually separated by one to three diameters ; middle half of disk with a narrow, indistinct, median longitudinal impunctate line; towards sides punctures become denser, slightly coarser, and broadly oval instead of round; surface between punctures with a hardly visible (mag. ×75) reticulate microsculpture and with a slight oily lustre. *Elytra* with intervals four to seven distinctly carinate from base to near apex, eighth similarly but less distinctly carinate, ninth only feebly carinate, second and third distinctly carinate except on basal fourth, and first (sutural) not carinate but with a sutural marginal line extending to basal fourth. *Abdomen* with sides or first four sternites two to three times as coarsely but only slightly more densely punctate than middle discal region. Fifth sternite with disk very nearly as coarsely and densely punctate as sides; apical marginal line nearly complete to base, more or less contiguous with apex. Tarsi of hind legs with first segment much shorter than combined length of two following (4 : 7).

FEMALE: Unknown. No obvious secondary sexual characters have been noted in the male.

TYPE: ♂, in the Museum of Natural History, Paris. Madagascar: near Marovoay, 1911 (*J. Descarpentries*).

COMPARATIVE NOTES: Its more completely divided eyes and unusual antennal structure will serve to distinguish it from its two Madagascan congeners.

Tribolium ceto sp. n. (figs. 29–30).

MALE: Length, 3·1–3·5 mm.; breadth, 0·9–1·0 mm. Body subparallel-sided, moderately convex. Cuticle feebly shining and dark reddish brown with antennae and legs much paler, brownish testaceous. Dorsal surface with setae just visible at a magnification of ×75. *Head* with punctures of middle between eyes round or oval, two-thirds to three-fourths as coarse as facets of eyes, and usually separated by less than one diameter, occasionally by as much as one diameter; surface between punctures with a dense reticulate microsculpture; margins above eyes not ridged. Clypeus with anterior margin, when seen from front, truncate or nearly so; surface more sparsely and much more finely punctate than head. Eye with narrowest part, where divided by side of front, as broad as five or six facets; eyes separated beneath head by less (6 : 8) than the greatest diameter of the ventral part of an eye, and space between eyes only slightly broader than postmentum (6 : 4·5); eyes only slightly but distinctly more prominent than sides of front. Antenna with an abrupt 5-segmented club, the seventh segment (first of club) being more than half again as broad as sixth; third segment slightly longer and distinctly narrower than second. *Pronotum* with broadest point, which is slightly before middle, broader than long (0·82 : 0·71 mm.) and base broader than apex (0·68 : 0·57 mm.). Sides arcuate and distinctly sinuate before hind angles, which are prominent (in two specimens the sides are scarcely noticeably sinuate at middle); sides and base completely margined, the basal margin gradually and slightly thickened towards middle; apical angles, when seen from above, not projecting quite as far forwards as middle of apex, which is unmargined. Disk with round to broadly oval punctures about two-thirds as coarse as facets of eyes and rarely separated by as much as one diameter but rarely confluent; towards sides punctures become as coarse as facets of eyes and are seldom separated by more than half of one diameter and are often contiguous or confluent; surface between punctures with an evident reticulate microsculpture but never with tubercles as in *T. thusa* sp. n. *Elytra* with intervals two to seven keeled from base to near apex; eighth and ninth intervals flat, but eighth sometimes feebly keeled for a short distance on apical third; first (sutural) distinctly margined only on apical third. Surface of discal intervals irregularly and sparsely rugose, with an evident reticulate microsculpture, but without distinct punctures. Discal strial punctures round, finer than those of pronotal disk, and separated longitudinally usually by three to four diameters. *Abdomen* with punctures of first four sternites nearly as coarse and dense on disk as on sides, those of disk of fifth sternite being nearly identical to those of sides ; marginal line of fifth sternite nearly complete to base and nearly contiguous to apex. Tarsi of hind legs with first segment as long as combined length of the two following segments.

FEMALE : Externally similar to male.

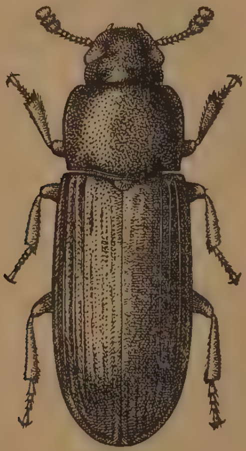
TYPE : ♂, in the Museum of Natural History, Paris. Madagascar, 1906 (*P. de la Bathie*).

PARATYPES : 2, with same data as type.

COMPARATIVE NOTES : From all other known species of *Tribolium* it may be distinguished by having the eyes slightly more prominent than the sides of the head. In general structure and in the close approximation of the eyes beneath the head it resembles *T. thusa* sp. n. of Africa but has not the ridged margins above the eyes of the latter species.

Tribolium freemani sp. n.

FEMALE : Length, 5·5 mm. ; breadth, 2·3 mm. Body subparallel-sided and moderately convex with disk of elytra nearly flat. Cuticle moderately feebly shining and moderately dark reddish brown. Dorsal surface with setae minute, scarcely visible at ×75. *Head* with most punctures oval, about two-thirds as coarse as facets of eyes and usually separated by half of one to one diameter, rarely confluent ; surface between punctures with a dense reticulate microsculpture. Clypeus with anterior margin shallowly, arcuately emarginate for its entire breadth ; surface of clypeus sculptured like middle of head but with punctures distinctly finer, more narrowly oval, and slightly but distinctly sparser than those of middle of head. Eye with narrowest part, where divided by side of front, as broad as two facets ; eyes separated beneath head by twice the transverse diameter of the ventral part of an eye (17 : 8), the space between the eyes being slightly more than twice as broad as postmentum (17 : 7). *Pronotum* at broadest point, which is slightly before middle, half again as broad as long (1·64 : 1·18 mm.) and base broader than apex



31

Fig. 31.—*Tribolium castaneum* (Herbst).

(1·53 : 1·01 mm.). Sides evenly arcuate, not distinctly sinuate before base; apex without a marginal line, the anterior angles, when seen from above, projecting slightly beyond apical margin; basal margin distinctly thicker at middle than at sides. Disk with punctures slightly coarser than those of middle of head, rarely oval, and separated by half of one to one diameter, rarely two diameters; basal half with a narrow, median longitudinal, impunctate line; sides with punctures about as coarse as facets of eyes, slightly denser than on disk, and round or broadly oval in shape; surface between punctures of all parts of disk with a dense reticulate microsculpture which is everywhere distinctly impressed. *Elytra* with intervals three to eight distinctly carinate from base to apex: ninth nowhere carinate; second indistinctly carinate near apex; and sutural (first) distinctly margined on apical fourth. Intervals punctate as in *T. castaneum*. *Prosternum* with deep, round to oval punctures about three-fourths as coarse as facets of eyes and separated by less than one to one (on sides) or two (on middle) diameters. Hypomera with punctures finer, sparser, and much more irregularly distributed than on prosternum, the surface between the punctures having a strongly impressed alutaceous microsculpture. Metasternum with sides punctate like pronotum and disk with punctures a third as coarse as facets of eyes and usually separated by three to five or more diameters. Abdomen with discal punctures of sternites slightly coarser than those of metasternal disk and mostly separated by two to three diameters; punctures of sides of sternites distinctly coarser and denser, the lateral punctures of first sternite being at least twice as coarse as discal; fifth sternite with marginal groove fine but distinct and complete nearly to base.

MALE: Unknown.

TYPE: ♀, in the British Museum (Nat. Hist.). Kashmir: Hispar, 189(3 ?) (*W. M. Conway*).

COMPARATIVE NOTES: This is very closely related to both *T. castaneum* and *T. madens*. From the former it may be distinguished by its much greater size (5·5 : 2·3–4·4 mm. long) and the much greater separation of the eyes beneath the head. From *T. madens* it differs as follows: it is larger; the cuticle is more or less uniformly reddish, whereas in *T. madens* it is black or nearly black and the antennae and legs are reddish or brownish and therefore strikingly different in colour from the cuticle of the dorsal and ventral surfaces; the narrowest part of the eye, where divided by the side of the front, is only as broad as two facets, whereas in *T. madens* it is as broad as four or five facets—usually five in the larger individuals; and the eyes are separated beneath the head by twice the transverse diameter of the ventral part of an eye (17 : 8), whereas in *T. madens* they are separated usually by three but sometimes by as little as two and a third diameters.

This species is named after Dr. J. Freeman.

Tribolium waterhousei sp. n.

MALE: Length, 4·7 mm.; breadth, 1·42 mm. Body subparallel-sided, moderately convex. Cuticle strongly shining and moderately pale reddish brown. Dorsal surface moderately sparsely clothed with inconspicuous, fine, erect, testaceous hairs about 0·05 mm. long or half as long as apical segment of antenna; hairs on surface of head and eyes shorter and more inconspicuous. *Head* with punctures round or irregular in shape, shallow, one-half to three-fifths as coarse as facets of eyes, and usually separated by two to four diameters on middle between eyes but occasionally by only one diameter; surface between punctures smooth (at × 75 mag.) and strongly shining. Clypeus with anterior margin very shallowly and broadly emarginate, nearly truncate; surface punctate like head but with the punctures distinctly finer, particularly anteriorly. Eye with narrowest part, where divided by side of front, as broad as 4½ facets; eyes separated beneath head by little more than the transverse (greatest) diameter of ventral part of an eye (13 : 10), the space between the eyes being twice as broad as postmentum (13 : 6). Antenna with club abruptly 3-segmented but with the seventh and eighth segments distinctly broader than the sixth. *Pronotum* at broadest point, which is slightly before middle, more than a fourth again as broad as long (1·39 : 1·04 mm.) and base broader than apex (1·34 : 1·01 mm.). Sides very feebly arcuate and slightly but distinctly constricted at basal sixth; apical angles, when seen from above distinctly projecting beyond apex, which is without a marginal line; base completely margined. Disk with punctures slightly but distinctly finer than those of middle of head and usually separated by three to five or more diameters; basal half of disk with an ill-defined median longitudinal impunctate line; surface elsewhere punctate like middle of head but with punctures less frequently round; surface between punctures everywhere smooth and strongly shining. *Elytra* without definitely formed keels or carinae but when viewed in certain lights with intervals three to six very indistinctly and feebly keeled; first (sutural) interval finely margined on apical third; discal intervals each with a single rather irregular row of very widely spaced punctures as fine as those of pronotal disk. Striae feebly impressed; strial punctures of disk not or only scarcely coarser than those of intervals and usually separated longitudinally by three to five diameters. *Prosternum* with moderately shallow oval or round punctures two-thirds as coarse, to as coarse, as facets of eyes and mostly separated by one to two diameters; punctures much finer, sparser, and less distinct on disk. Hypomera with punctures sparser and slightly finer than those of sides of prosternum and surface also feebly rugose. Metasternum with punctures of sides two-thirds as coarse as facets of eyes and separated by one to two diameters, but much finer and sparser near posterior margin; disk with punctures as fine as those of pronotal disk and separated by three to ten diameters. Abdomen with punctures of sides of first two sternites similar to those of metasternal sides but sides of apical sternite much more finely and sparsely punctate; discal region of sternites with punctures, decreasing gradually in size and density from the first to the fifth, the punctures of the first sternite being as fine as those of metasternal disk and usually separated by three to five diameters; fifth sternite without an apical marginal line.

FEMALE: Externally similar to male but without the pit and associated brush of hairs on the basal part of the ventral side of the front femur.

TYPE: ♂, in the British Museum (Nat. Hist.). Australia: New South Wales; Bombala, ii. 1939 (*C. J. Mann*).

PARATYPE: ♀, Australia: Queensland, Rockhampton.

COMPARATIVE NOTES: This is closely related only to the species described below from which it may be distinguished as shown under the heading of the latter. I take pleasure in naming this species after Mr. Douglas Waterhouse.

Tribolium parki sp. n.

FEMALE: Length, 3·9 mm.; breadth, 1·09 mm. Externally similar to *T. waterhousei* from which it may be distinguished as follows: (1) smaller, 3·9 : 4·7 mm. long; (2) cuticle browner with much less red, the legs being brownish testaceous instead of reddish; (3) punctures of middle of head round or nearly so, relatively deeper and more distinct, and mostly separated by one or slightly less than one diameter but occasionally by two diameters; (4) pronotum slightly more convex and narrower, the breadth/length ratio being 1·13 instead of 1·34; (5) sides of pronotum evenly narrowed to base and not slightly constricted at basal sixth; (6) antero-ventral margin of head opposite base of maxilla only slightly produced, the produced part being no longer than third antennal segment, whereas in *T. waterhousei* the produced margin opposite the base of the maxilla is in the form of a very stout rounded tooth about as long as third and fourth antennal segments together; (7) discal punctures of first abdominal sternite no coarser or denser than those of fourth sternite, whereas in *T. waterhousei* the discal punctures of the first sternite are about twice as coarse and dense as those of the fourth ; and (8) apex of fifth sternite with a fine marginal line which, however, is indistinct.

MALE : Unknown.

TYPE : ♀, in the British Museum (Nat. Hist.). Larat Island (*F. Muir*).

This species is named after Dr. T. Park who has contributed so much to our knowledge of the biology of various species of *Tribolium*.

Tribolium cylindricum sp. n.

MALE : Length, 3·19–3·59 mm. ; breadth, 0·82–0·90 mm. Body narrow, parallel-sided, rather strongly convex, subcylindrical. Cuticle strongly shining and very dark reddish brown, nearly black ; antennae and legs considerably paler and reddish brown, the legs being more red than the antennal club. Dorsal surface with indistinct, fine, sparse, erect, yellowish hairs which on elytra are about as long as apical segment of antennal club but on pronotum and eyes are considerably shorter. *Head* on middle with round to broadly oval punctures which are about one-half to two-thirds as coarse as facets of eyes and are usually separated by half their diameters but are occasionally longitudinally confluent ; longitudinally confluent punctures never sufficiently numerous to make the surface appear longitudinally rugose ; surface between punctures smooth and strongly shining. Clypeus with anterior margin broadly and moderately shallowly, arcuately emarginate ; surface with punctures slightly finer than those of middle of head and towards sides considerably sparser, being here as sparse as those of sides of head. Antenna with a very abrupt 3-segmented club but with segments seven and eight distinctly broader than sixth. Eye with narrowest part, where divided by side of front, as broad as 4½–5 facets ; eyes separated beneath head by the transverse diameter of ventral part of an eye or by little more (10 : 8), and space separating eyes twice as broad as postmentum (10 : 4·5). *Pronotum* at broadest point, which is at about middle, scarcely broader than long (0·85 : 0·82 mm.) and base slightly broader than apex (0·76 : 0·68 mm.). Sides feebly arcuate, nearly straight, and area near sides so convex that most of the lateral margin is concealed from above ; base completely and distinctly margined ; apex, when seen from above, slightly arcuate and projecting further forwards than anterior angles, without a marginal line. Disk with punctures slightly but distinctly finer than those of middle of head and usually separated by one to three diameters, but in some specimens most punctures are separated by two to four diameters ; on sides and extreme base punctures are coarser and denser, on sides being oval, slightly coarser than those of head, and usually separated by slightly less than one to one diameter ; punctures everywhere ocellate (with minute central puncture) but on sides very distinctly so ; surface between punctures everywhere smooth and strongly shining . *Elytra* with intervals flat or nearly so, but when seen in some lights intervals four to eight have a fine, indistinct keel on basal third. Striae very distinctly impressed ; discal strial punctures nearly as coarse as those of pronotal disk and usually separated by two to three diameters ; punctures of intervals sparser and slightly finer than those of striae ; surface also with widely and irregularly spaced, fine, transverse wrinkles but otherwise smooth and strongly shining. *Prosternum* with round punctures as coarse as those of pronotum but deeper and separated by one to two diameters. Hypomera much more sparsely punctate and with a distinct microsculpture between the punctures. Metasternum with punctures of sides similar to those of prosternum but shallower ; disk with punctures much finer and sparser than those of sides. Abdomen with punctures of sides of sternites similar to those of metasternum but becoming finer and sparser on apical sternites ; discal area of all sternites with punctures sparser and distinctly finer than those of sides ; fifth sternite with apical marginal line very fine and close to apex.

FEMALE : Externally similar to male but without a pit and associated brush of hairs on ventral basal part of front femur.

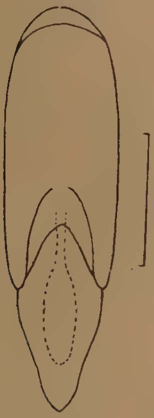
TYPE : ♂, in the British Museum (Nat. Hist.). Malay Peninsula : Singapore, iii. 1922 (*C. J. Saunders*).

PARATYPES : 7, Singapore, 1921–23 (*J. C. Saunders*) ; 1, Singapore (C. Lewis collection) ; 1, Malay Peninsula : Kuala Lumpur, Cheras Road, 30.x.1922 (*H. M. Pendlebury*) ; and 7, Moluccas : Batchian (*A. R. Wallace*).

COMPARATIVE NOTES : This species is relatively narrower and more parallel-sided than any other member of its genus. It is unique in having the prothorax approximately as long as broad, the breadth/length ratio being 1·03. In all other species the prothorax is evidently broader than long, the breadth/length ratio varying from 1·1 to 1·4. A further unusual feature of the prothorax is that the sides are sufficiently convex to conceal most of the lateral margins from a dorsal view.

Tribolium politum, sp. n. (fig. 32).

MALE : Length, 4·5 mm. ; breadth, 1·78 mm. Body subparallel, moderately convex. Cuticle strongly shining, highly polished and moderately dark reddish ; antennae brownish testaceous. Surface of eyes with numerous erect, testaceous hairs which are about as long as an eye facet ; surface of elytra sparsely clothed with fine, erect, testaceous hairs which are slightly longer than apical segment of antenna. *Head* and clypeus with sparse microscopic punctures about a seventh as coarse as facets of eyes ; surface between punctures highly polished, without trace of microsculpture (at mag. ×75). Clypeus with anterior margin shallowly and broadly, arcuately emarginate. Eye with narrowest part, where divided by side of front, consisting of five rows of facets ; eyes separated beneath head by the transverse diameter of ventral part of an eye, and space separating eyes equal to twice the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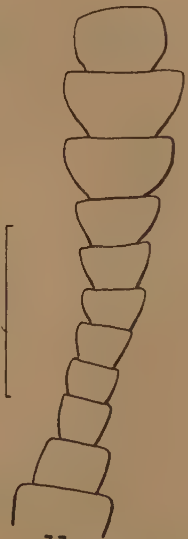
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Fig. 32.—*Tribolium politum* sp. n. Dorsal view of male genitalia.
Fig. 33.—*Tribolium antennatum* sp. n. Antenna.

breadth of the postmentum (12 : 6). Antenna with a very abrupt 3-segmented club but with segments six to eight increasing progressively in size. *Pronotum* at broadest point, which is at about middle or slightly before, half again as broad as long (1·72 : 1·12 mm.) and base broader than apex (1·64 : 1·12 mm.). Sides evenly arcuate; apex not margined even at extreme sides; base completely margined, the marginal line being nearly twice as thick at middle as at sides. Surface evenly convex; microscopically punctate and highly polished like head. *Elytra* sculptured like pronotum, without striae or carinae; punctures from which setae arise slightly larger than others; suture faintly margined on apical fourth. *Prosternum* with punctures at sides one-half to two-thirds as coarse as facets of eyes. Hypomera with fine and irregular, longitudinal strigae. Metasternum and abdomen with discal area punctate like pronotum but very slightly more coarsely so; sides of first two abdominal sternites with many punctures about three times as coarse (nearly half as coarse as facets of eyes); fifth sternite with marginal line present only at sides, where it is indistinct.

FEMALE: Unknown.

TYPE: ♂, in the collection of the British Museum (Nat. Hist.) Riouw Arch.: Doerian Island, xi.1923 (*Dammerman*).

COMPARATIVE NOTES: This is the only species of the genus which has all the punctures of the dorsal surface very much less than half as coarse as the facets of the eyes. It is the only species without either striae or carinae on the elytra.

Tribolium antennatum sp. n. (fig. 33).

FEMALE: Length, 3·5 mm.; breadth, 1·18 mm. Body subparallel-sided, moderately convex. Surface of eyes, margins of pronotum, and all of dorsal surface of elytra with sparse, erect, testaceous hairs which are about two-thirds as long as apical segment of antenna. *Head* with round to oval, shallow punctures which are nearly as coarse as facets of eyes and are separated by considerably less than one diameter but are seldom confluent; sides between anterior angles of clypeus and projection over eye very broadly, shallowly, arcuately emarginate. Clypeus with anterior margin shallowly, arcuately emarginate for nearly all of its breadth; surface with punctures about half as coarse as those of middle of head and usually separated by one to two diameters; surface between punctures with a distinct (mag. ×75) but lightly impressed reticulate microsculpture. Eye with narrowest part, where divided by side of front, as broad as four facets; eyes separated beneath head by 1½ times the transverse diameter of the ventral part of an eye (11·5 : 7·5), the area between the eyes being broadly, moderately deeply concave and twice as broad as postmentum (11·5 : 5). Antenna with club (fig. 33) strongly flattened and apical segment shorter and much narrower than penultimate. *Pronotum* at broadest point, which is at about middle, much broader than long (1·01 : 0·68 mm.) and base broader than apex (0·98 : 0·73 mm.). Base without a marginal line on middle two-thirds. Disk with punctures about as coarse as facets of eyes or very slightly coarser, round or nearly so, and mostly separated by less than one diameter though never confluent, but occasionally separated by as much as one diameter; at sides the punctures are slightly but distinctly coarser, usually oval instead of round, and very slightly denser than on disk; surface between punctures with a very lightly impressed reticulate microsculpture. *Elytra* with intervals two to nine finely and distinctly carinate from near base to near apex; first interval with marginal line of suture present only on apical third. *Prosternum* with punctures as coarse and dense as those of pronotal disk but much deeper. Metasternal sides punctate like sides of pronotum but slightly more sparsely so; disk with about 40 punctures as coarse as facets of eyes and separated usually by one to two diameters; transverse impression before hind coxa curved and deeper than usual in the genus. Abdomen without a marginal line on fifth sternite; sides of first two sternites punctate like sides of metasternum, the discal punctures of these two sternites being slightly finer but scarcely sparser; sternites three to five with punctures rapidly decreasing in size so that on fifth they are only about a fourth as coarse as on first.

MALE: Unknown.

TYPE: ♀, in the British Museum (Nat. Hist.). Australia: Queensland (*F. P. Dodd*).

COMPARATIVE NOTES: The chief differences between this species and *T. myrmecophilum* have already been listed in the key.

References.

ANDRES, A. (1931). Catalogue of the Egyptian Tenebrionidae.—Bull. Soc. ent. Egypte, **15**, pp. 74–125.

DU TOIT. (1937). Our wandering continents (p. 125). Edinburgh.

GOOD, N. E. (1936). The flour beetles of the genus *Tribolium*.—Bull. U.S. Dep. Agric., no. 498, 57 pp. 22 figs.

HINTON, H. E. (1945). A monograph of the beetles associated with stored products. Volume I.—London, Brit. Mus. (Nat. Hist.) viii+443 pp. 505 figs.

MATTHEW. (1915). Climate and evolution (p. 35).

UYTTENBOOGAART, D. L. (1934). Revision des Genus *Tribolium* (Col. Ten.).—Ent. Bl., **30**, pp. 20–31.

THE BIOLOGY AND CONTROL OF THE PEA MOTH, *LASPEYRESIA NIGRICANA*, STEPH.

By D. W. Wright, M.A. and Q. A. Geering, B.A.
Horticultural Research Station, Cambridge, and School of Agriculture, Cambridge.

(Plate IX.)

Contents.

	PAGE
Introduction	57
Synonymy	57
Biology	57
Parasitism	67
Cultural Control	69
Chemical Control	72
Acknowledgements	83
References	84

Introduction.

The Pea Moth, *Laspeyresia nigricana*, Steph., is one of the principal pests of garden and field peas in Great Britain. Its larvae feed within the pods causing considerable damage to the developing seeds and giving to their contents a very unsightly appearance. The insect is widely distributed in England and, according to Meyrick (1927), occurs in Scotland as far north as the Clyde. It is most prevalent, however, in the southern part of England, where in the pea growing counties of Lincoln, Essex and Kent it frequently causes severe losses. It is widely distributed in central and southern Europe while in North America it has spread, since its introduction, to all the main pea producing areas and has become a major pest.

The observations and experiments described in this paper were chiefly carried out in the Harlow district of Essex and at the Horticultural Field Station, Cambridge. In both localities the moth is very prevalent. The investigation was begun in 1945 and continued during 1946 when most of the data were obtained.

Synonymy.

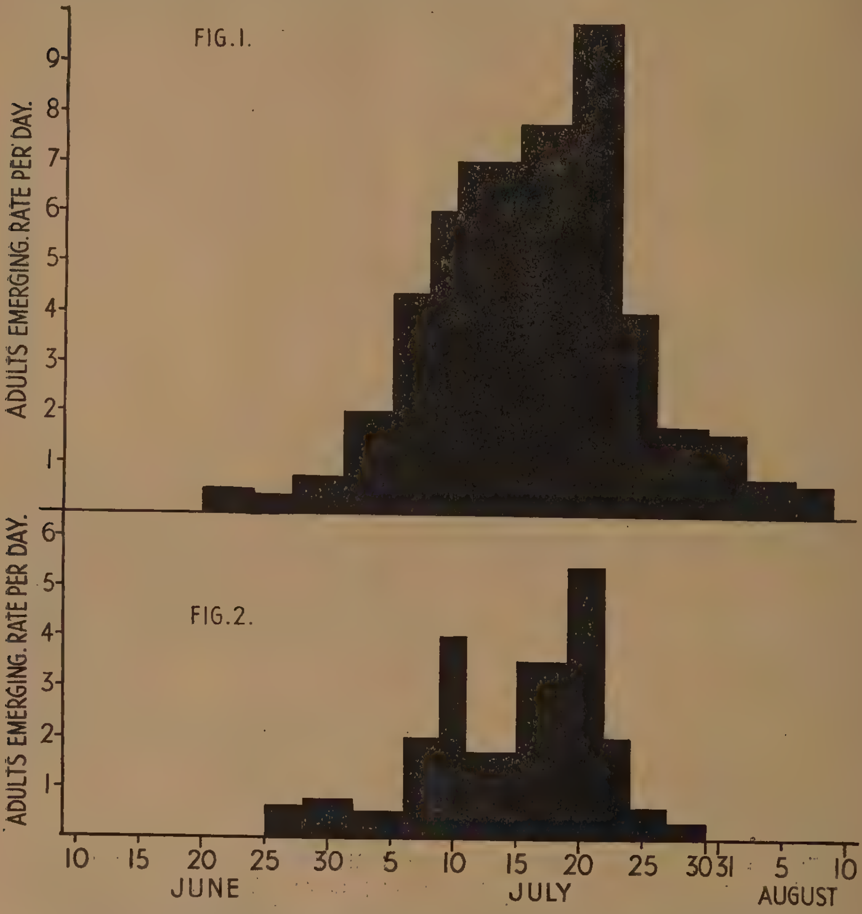
The name "Pea Moth" has been given to 3 distinct species of the genus *Laspeyresia* Hübner. The only species found feeding on peas in this investigation was *Laspeyresia nigricana*, Stephens. The more important synonyms of this species are, *Cydia nigricana*, Steph., *Laspeyresia pisana*, Guen., *L. proximana*, Wilk., *Grapholitha nigricana*, Tr., *Endopisa nigricana*, Steph., and *Enarmonia nigricana*, F.

Biology.

Emergence of the moths.

Data on the period of emergence of the moths were obtained by erecting cages on the site of pea stacks harvested in the previous year. At Harlow emergence began in mid-June 1945 and continued until July 25 whilst in 1946 it began a little later, on

June 20, and continued until August 9; at Cambridge in 1946, it occurred from June 25 to July 30. The emergence in both localities for 1946 is shown in figs. 1 and 2. In both seasons moths were taken in the field about a week before they were recorded in the cages. This appeared to be due to the insulating effect of the cages causing the temperature of the soil inside to be lower than that outside. The true peak period of emergence, therefore, predates that shown in the records by about one week. This was confirmed by estimates of field abundance.



Figs. 1–2.—(1) Emergence of Pea Moth from a Stack Site at Harlow, Essex, 1946.
(2) Emergence of Pea Moth from the Field Station, Cambridge, 1946.

Abundance of moths in the field.

The assessment of the abundance of insects in the field is often a difficult task, largely on account of interacting factors which combine to limit their availability. This is particularly so with the pea moth which shelters deeply in the vegetation and when disturbed usually flies well below the crop level. Trapping with molasses baits and by sticky bands in 1945 gave poor results and proved generally unsatisfactory.

Considerable numbers can, however, be taken by sweeping but the proportion of the population taken appears to vary both with the type of crop swept and with the activity of the moths. The sheltering moths are most difficult to sweep on a pea crop, but when in flight they can be taken at crop height by swift strokes of the net ; they may be readily swept in a potato crop in both circumstances as the net can be used between the rows well below crop level.

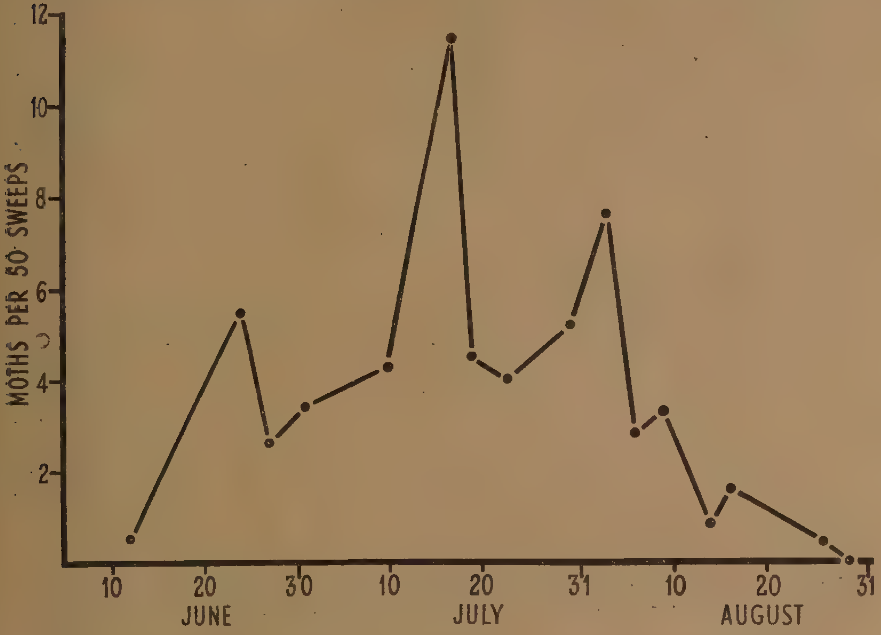


Fig. 3.—Field abundance of adult Pea Moths, on a potato crop following seed peas. Harlow, Essex, 1946.

The sweeping results shown in fig. 3 were obtained from a field of potatoes which had grown peas for seed in the previous year. The graph shows the trend of the population over the period, but the fluctuations in numbers obtained was due primarily to the effect of environmental conditions on the availability of the moths.

TABLE I.

Effect of sunshine and temperature on the activity of pea moth on a July afternoon, 1946.

Time G.M.T.	Sunshine	Temperature °F.	Activity
2.30	Full	65	Nil
3.00	Full	66	Very Active
4.00	¼ Full	65	Very Active
5.00	¼ Full	64	Nil
5.05	Full	66	Very Active
5.10	Nil (Overcast)	63	Active
5.12	Nil (Overcast)	62	Nil
5.15	Full	66	Active

Sweeping also showed that there was no marked tendency of the moths to concentrate in or near hedges or other windbreaks. They were, however, more common in dense than in sparse vegetation.

Behaviour of moths in the field.

Data were collected concerning the factors affecting the activity of the moths in the field. Moths were " active ", *i.e.* flying on their own account, only after 2 p.m. ; they were always inactive before this time, *i.e.*, not flying, or flying only for a short distance if disturbed. The moths became active in full sunshine when the air temperature reached 63°F. but with the sun more than half obscured by cloud the lower limit of activity was approximately 65°F. Activity occurred during full cloud but a temperature limit was not obtained. The relationship between activity and temperature, as influenced by cloud, is shown by the data obtained on a July afternoon and is given in Table I. As the sun became obscured and the temperature fell below 65°F. the moths began to settle and activity ceased before 62°F. had been reached.

The moisture content of the air (within the limits observed) appeared to have little effect on activity. Similarly normal flight occurred at windspeeds of 0–4 m.p.h. (estimated at crop height). The main period of activity was usually between 4 p.m. and 6 p.m. G.M.T.

In the summer of 1946 unusually wet weather conditions occurred during the flight period of the moths. The suitability of these conditions for flight activity was assessed from data obtained from the Meteorological Station at Writtle, Essex. Records taken here at 4 p.m. G.M.T. showed that on 39 of the 55 days concerned (June 20–August 14) air temperatures of 65°F. or higher were reached. The infestations in crops in this area were considerable ; an 11-acre pea field, situated half a mile from the nearest pea crop of the previous year received an influx of moths sufficient to produce a 20 per cent. pod infestation. These attacks showed that conditions over much of the flight period were favourable for activity, as was also indicated by the temperature data.

Preoviposition period.

Under insectary conditions the time from the emergence of the females to oviposition varied from 5 to 11 days, the average period being 8 days. This corresponds closely with the observations of Baker (1937), whilst Langenbuch (1941) found the period to vary between 5 and 13 days with an average of 9 days.

The egg stage.

The egg is very flattened, oval in shape and measures 0·75 mm. by 0·53 mm. The incubation period varies according to the temperature. Under controlled conditions, at temperatures of 59°F., 68°F. and 77°F. it lasted 9, 6 and 3 days respectively. In the field the time varied between 6 and 11 days according to the weather, and averaged 7–8 days. The eggs are whitish and semi-translucent when newly laid. After 2 to 3 days two pink streaks develop ; these disappear on the 6th or 7th day when the outline of the larva, particularly the head capsule, can be seen.

Only the upper half of the pea plant is apparently selected for oviposition, and egg distribution seems to be at random ; the moths show no special preference to oviposit on or near the flowers or pods. This habit of the adult has also been noted by other workers. The distribution on the plant as observed in 1946 is shown in Table II.

Under laboratory conditions moths oviposited freely on pea and other foliage and, when these were absent, on the walls of the container. In pea crops eggs were only found on pea plants and were not found in fields where the plants had not begun to flower. Eggs were found, however, on individual non-flowering plants occurring amongst others which were in flower.

TABLE II.

Location of eggs on pea plants 1946.

Position		Percentage
Leaf	under-surface	51·5
,,	upper-surface	13·6
Stipule	under-surface	15·2
,,	upper-surface	4·5
Petiole	under-surface	4·5
,,	upper-surface	—
Petal		—
Sepal		9·1
Pod		1·5

The shortest distance along the plant between the eggs and the nearest flower or pod was also recorded; in the semi-dwarf varieties, Kelvedon Wonder and Onward, the distance ranged from 0 to 9 inches with an average of 4·3 inches. In the case of the tall varieties the distances involved were 0 to 12 inches with 7·0 inches as the average.

The larva.

The newly hatched larva is whitish in colour except for a brown head and a brown dorsal shield on the first thoracic segment. It measures about 1·2 mm. long and is 0·2 mm. broad in the thoracic region. The first-instar larva is very active and individuals have been observed to crawl over pea plants, apparently in a random fashion, for 24 hours before entering a pod. The hole bored by a larva through the pod wall heals in a few days to give a small cork-coloured blister. Pods at all stages of development, until they begin to ripen and become parchment-like, can be penetrated by the larvae. According to Langenbuch they do not swallow the epidermis of the pod, and this would account for the ineffectiveness of stomach poisons for the control of this pest.

The larva feeds on the young seeds inside the pod, damaging from 1 to 6. In 300 mature pods each attacked by a single larva the average number of seeds damaged was 2·4. Usually one or two seeds are badly attacked while others show less damage. The extent of the damage on each seed decreases as the number of seeds attacked increases.

The period spent inside the pod, until the larva is fully fed, appears to vary according to the temperature. Under insectary conditions in 1946 the time was from 18 to 30 days, the longer period occurring towards the end of the season. The larva measures 12–14 mm. in length when fully fed. It leaves through a hole cut in the pod and passes into the soil.

Larval populations in the field.

The growth and changes in the larval population on a pea field in 1946 were followed by examining samples taken at intervals after the commencement of the attack. The larvae in each sample were graded into three groups, " small ", " medium " and " large ", corresponding to length measurements of 0–3·5 mm., 3·6–6·0 mm. and 6·1 mm. and over, respectively. Record was also made of those which had matured and cut out of the pods. The changes in composition of the population over the period is shown in fig. 4. In early September, when but for very wet weather conditions the crop would have been stacked, 46·5 per cent. of the larvae were still in the pods. The crop, however, was not harvested but a number of whole plant samples were taken and placed in an insectary. Here they dried out and reached a condition comparable to that occurring towards the outside of pea stacks. Samples were examined at intervals and by the end of September 93·6 per cent. of the total population had matured and left the pods. Thus in this crop 53·5 per cent. of the total larval population were present when the crop should have been harvested but at the end of a month under stack conditions only 6·4 per cent. remained in the pods. This shows the manner in which large larval populations are built up on stack sites (*vide infra*). Under normal conditions of harvesting only 10–15 per cent. of the total larval populations are carried with the crop to the stack sites.

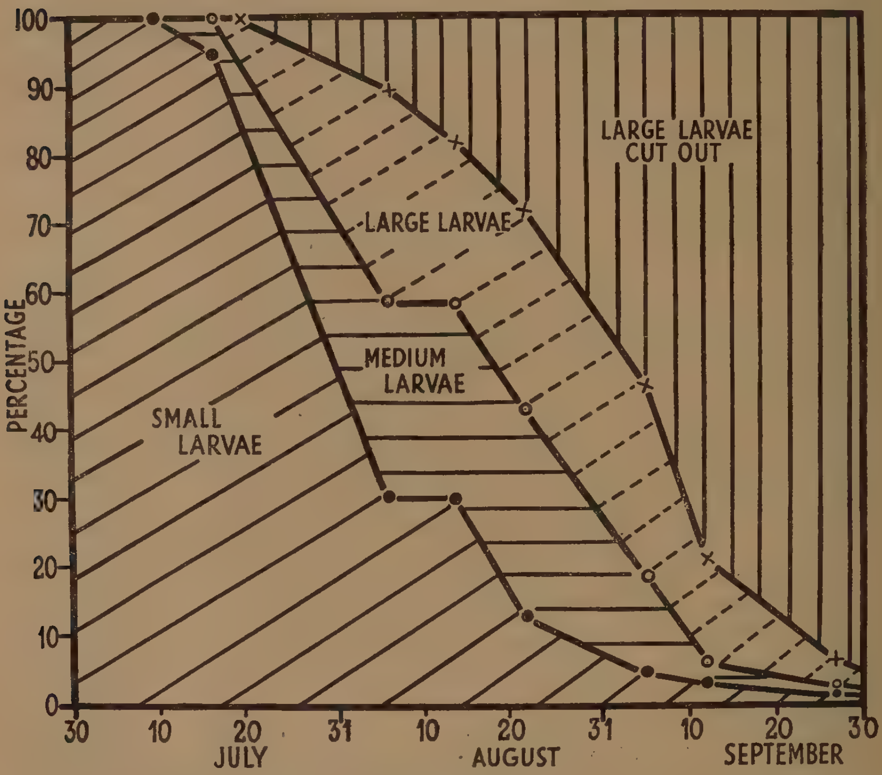


Fig. 4.—Changes in composition of larval population from July to September. Harlow, Essex, 1946.

The development of the total larval population in the field crop is shown in fig. 5 (curve a) and is there compared over the same period with the percentage of pods attacked (curve b). The attack reached its maximum at the end of August. The divergence of the two curves, which increases progressively with increase in the attack, is due to young larvae entering both unattacked and previously attacked pods at random. This is shown by the average number of larvae occurring per pod which on

17 July was 1·12 and on 22 August was 1·34. The maturation of the larvae in the pods is shown by curve (c). Except at the two extremities it closely parallels curve (a) and reflects the growth in numbers and in age of the population.

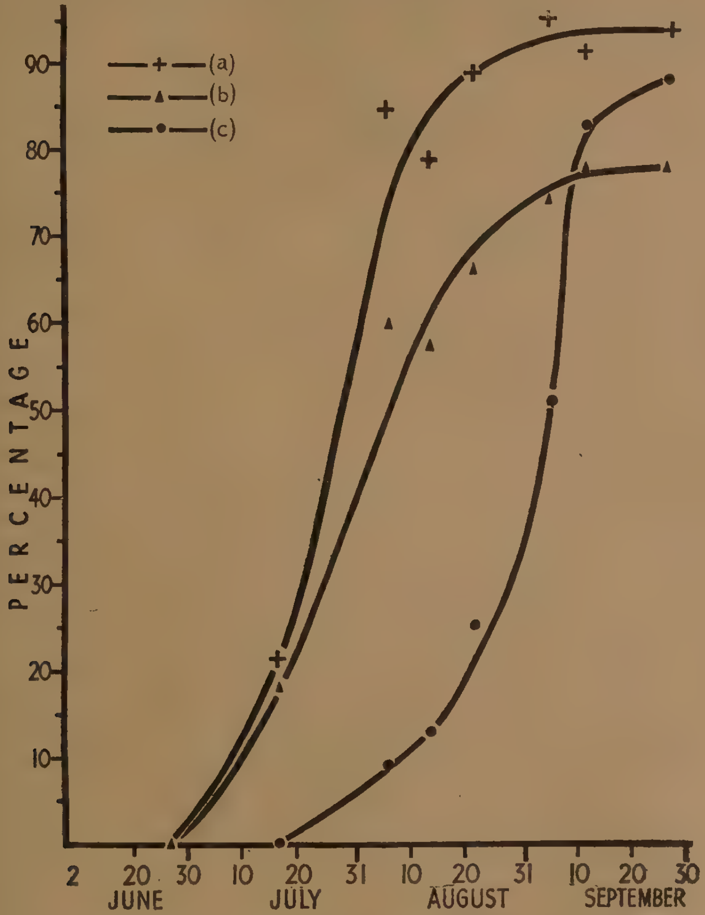


Fig. 5.—Field population of larvae, Harlow, Essex, 1946. (*a*) Total larvae per 100 pods; (*b*) Percentage of pods attacked; (*c*) Larvae cut out per 100 pods.

The effect of the drying out of the pods and peas on the immature larvae.

A small percentage of the larvae fail to leave the pods when pea crops are harvested for seed. These are chiefly immature individuals which have been prevented from further feeding by the hardening of the seeds. In 1946 the behaviour and fate of these larvae in the dry pods were followed. Samples, each containing 20 whole plants, were collected in the field on August 26 and transferred to an unheated insectary where conditions were similar to those occurring in a stack. These were examined at intervals throughout autumn and winter and the number and condition of the larvae and their location in the pods were noted. The results are given in Table III.

It will be seen that a considerable mortality occurred during the autumn, the very small larvae succumbing first, followed by the less immature forms. Death was apparently due to starvation and desiccation and no larvae in the 3rd instar or younger appeared to survive. The remaining older larvae were found either inside the seeds or in the pods. A few of those in the pods spun their cocoons but by December they had either shrivelled and were dead or had disappeared. Larvae were found inside the seeds in fairly constant numbers from September onwards; each had eaten a large cavity in the seed and had surrounded itself with a cocoon. Here they were found in a healthy condition until the spring. Although no tests have been carried out, if such seeds were sown it seems most probable that the larvae would be able to complete their development and transform into moths. Two healthy larvae were found inside peas in a commercial seed sample in March 1946. This would appear to be a method whereby this insect can be and probably has been transported to new districts and continents.

TABLE III.

The effect of drying out of the pods on the remaining larval population.

Date of Examination	No. of larvae inside peas		No. of larvae free inside pod		Total living larvae per sample	No. of living larvae inside peas (per 1,000 seeds)
	Alive	Dead	Alive	Dead		
August 26	0	0	18	4	18	0
September 12 ...	3	0	9	1	12	6·5
September 27 ...	2	0	3	0	5	4·3
October 12... ...	3	0	3	10	6	6·9
October 29... ...	5	0	5	1	10	12·1
November 15 ...	1	0	3	3	4	2·0
December 6 ...	4	2	0	0	4	11·8
March 12 ...	4	2	1	1	5	10·8

Overwintering populations of larvae.

The overwintering larvae occur principally in two situations: on fields where pea crops have been grown, and where peas harvested dry have been stacked. Fields where peas have been grown for seed necessarily carry a higher population than those where the crop has been picked green, since in the latter case the majority are removed when the crop is picked. At the time of green picking, however, a considerable number of the larvae have already matured and left the pods and very large numbers of pods are overlooked by the pickers. From samples taken in 1945–46 on a number of recently picked fields the pod and larval populations remaining per acre were calculated and are shown in Table IV.

TABLE IV.

Population of pods and larvae occurring after crops picked green.

Field		No. of pods per acre unpicked	Total larval population per acre in unpicked pods
1945 ...	1	203,000	23,000
	2	180,000	7,300
	3	215,000	21,200
1946 ...	1	189,900	62,400
	2	217,800	67,400

The data show that peas when picked green are by no means a self cleaning crop in respect of pea moth. Futhermore, placing the haulm in small cocks in the field to dry allows most of the remaining larvae to finish feeding and enter the soil. Very high concentrations of larvae were found under such cocks and these were not destroyed by subsequent burning of the cocks.

Much greater populations are liable to occur than those recorded above in fields where the peas have been harvested dry for seed. In a field trial at Harlow in 1946 the total larval populations at the harvesting stage on the varieties Onward and Lincoln Blue were 607,000 and 1,020,000 per acre respectively. Some 300,000 and 685,000 per acre of these respectively were mature and had left the pods by the time the crop was cut, and many more followed before stacking.

The proportion of the total population which is transferred to the stack varied from 10 per cent. to approximately 50 per cent. ; the percentage tends to decrease the longer the crop is left in the field to dry. A study of the soil under pea stacks has shown that these sites usually contain great numbers of larvae and are a potent source of pea moth. The highest concentration of larvae found was 1,737 per square yard under a stack of the variety Maple. The larvae were distributed over the whole stack bottom but were more highly concentrated towards the edge.

Behaviour of larvae in the soil.

After leaving the pod the larva enters the soil and spins around itself a thick silken web or cocoon. It is oval in shape and measures approximately 10 mm. by 4·5 mm. Small particles of soil adhere firmly to the surface so that it is very difficult to distinguish from a small lump of earth. The cocoon appears to be waterproof and thus protects the larva from waterlogging. Stratification samples were taken during autumn, winter and spring on a heavily infested plot in order to obtain information on their depth and distribution in the soil. These showed that 92·6 per cent. of the cocoons occurred in the top 2 inches of soil, 4·7 per cent. in the next two inches, whilst the remainder, 2·7 per cent. were scattered in the soil from 4 to 10 inches in depth. The larvae remained quiescent throughout the winter in their cocoons, but in April those at the lower depths in the soil began to leave their cocoons and came into the top two inches of soil where they formed a second cocoon inside which pupation occurred. A proportion of the larvae which had passed the winter near the soil surface appeared also to form second cocoons. These cocoons are thin and web-like and are much longer than those formed in the autumn ; they occur immediately below the soil surface. The capacity of the larvae to move through the soil was tested by placing cocoons at levels varying from 2 inches to 24 inches below the soil surface. This showed that from the depths of 12 inches or less they were able to emerge successfully, but from 24 inches a high mortality occurred and only a few larvae covered the distance.

Samples taken from stack sites in the summers of 1945–46 indicated that in each year a small percentage of the larvae passed through a 2-year life cycle, having spent two winters and a summer in the soil before pupating. These larvae appeared identical with those which had the normal cycle and were probably individuals in which for some reason unknown the winter diapause had not been broken (*vide infra*).

The effect of soil temperatures on the development of the overwintering larvae.

The effect of soil temperature on the development of the overwintering larvae was investigated with a view to determining its influence on the number of generations produced in a year and on the rate of larval development. According to Hanson and Webster (1936) a partial second generation occurs in the State of Washington, and it would appear possible that under mild autumn conditions this might also occur in Great Britain. Nicolaisen (1928), and Langenbuch (1941) obtained no evidence that in

Germany a second generation was produced under natural conditions. Both workers, however, bred small numbers of moths from larvae which had recently left the pods. The larvae were induced to pupate prematurely by keeping them under very unnatural conditions in the laboratory.

In the present investigation the capacity of the overwintering larvae to continue development was tested under various temperature conditions. Larvae were taken from a field in September and subjected to a constant temperature of 20°C. (68°F.) for 6 months. They remained healthy throughout but no further development occurred. Larvae were taken from the same field in January and placed at a constant temperature of 15°C. (59°F.). Moths began to emerge 56 days later. The principal difference between the two sets of larvae was that those collected in September had not been subjected to a temperature below 12°C., while those collected in January had been exposed to outside soil temperatures of 5°C. and lower for a considerable time. This and other experiments indicate that after leaving the pods the larvae enter a diapause. Under natural conditions this is broken by a period of low temperature such as normally occurs during an English winter. Under laboratory conditions it appears possible to break the diapause by other means. The results, therefore, indicate that the physiological condition of the larvae in the autumn is such that a second generation of moths will not normally occur under the temperate climatic conditions of Great Britain.

When the diapause has been broken the rate of development in spring and the time of the emergence of the moths appears to be governed by soil temperature.

Larvae were subjected to a series of temperatures corresponding to those occurring in the soil in a warm spring and these gave rise to moths within 80 days of the beginning of the experiment. Other larvae were subjected to a series of lower temperatures corresponding to a cold spring ; in this case 118 days were required before moths were produced.

The effect of prolonged low soil temperature on the mortality of the larvae was also investigated. Larvae in soil were subjected to −5°C. (23°F.) for 17, 24 and 31 days. After this they were placed at a temperature suitable for development and in all cases moths were produced. Not one of the treatments appeared to have increased mortality.

The pupa.

The pupa is formed inside the cocoon in a position just below the soil surface. It is dark brown in colour and measures 7–8 mm. in length and about 1·7 mm. in maximum breadth. On the dorsal surface of each abdominal segment it carries two rows of spines, except on the last three segments which have only a single row each. The spines of the front row are fewer and stronger than those of the second row. There is a blunt cremaster which also has 6–8 spines (Pl. IX). The spines are strongly reflexed and assist the pupa in pushing itself out of the cocoon to the soil surface, where it lies partly or fully exposed when the moth is ready to emerge. Frequently the last abdominal segments are not fully withdrawn from the cocoon.

In 1946 pupae were first found in the field on June 3 and occurred henceforth until July 9. The length of the pupal stage was 12–14 days under field conditions.

Other hosts of the Pea Moth.

Larvae, identified as those of the Pea Moth, have been obtained from the following wild and cultivated plants. The pod infestations given in Table V are the maximum recorded in 1946.

TABLE V.

	Per cent. pod attack
Lathyrus pratensis, Linn. (Meadow pea) ...	26·5
Vicia Cracca, Linn. (Tufted vetch)	12·5
Lathyrus odoratus, Linn. (Sweet pea) ...	3·0
Vicia sativa, Linn. (Field vetch)	0·28

Both wild vetches are common in the Harlow district of Essex and serve as a natural breeding ground for the pea moth. In this district, however, the population bred on cultivated peas must greatly outnumber that from all other sources.

Parasitism.

The following three Hymenopterous parasites were reared from the larvae of the Pea Moth.

Ascogaster quadridentatus, Wesm. (Braconidae).
Glypta haesitator, Grav. (Ichneumonidae).
Hemiteles ridibundus, Grav. (Ichneumonidae).

Both *A. quadridentatus* and *G. haesitator* have been described in some detail by Cameron (1938). In the former the eggs are laid within those of the Pea Moth and develop within the larva of the host; the latter is also a larval parasite but the hosts are attacked when in the pods. No information is available concerning the biology and habits of *H. ridibundus*; its long ovipositor indicates that it probably behaves similarly to *G. haesitator*. The adults of the three species emerged during the emergence period of the Pea Moth and were trapped in the cages erected for the latter. The emergence period of each parasite is shown in fig. 6, the data being obtained in 1946. The incidence of attack by each of the parasites in the pea moth larvae in three stack sites at Harlow and on a field plot at Cambridge is shown in Table VI. All three species were common in each locality and together caused considerable mortality in the host population. Estimates of incidence of parasitism based on soil samples were very similar to those given by the numbers of parasites and hosts emerging on the same sites. This close agreement indicated that few parasites or moths escaped from the cages.

TABLE VI.

Percentage parasitism of the Pea Moth, 1946.

Locality	Site	*A. quadridentatus* per cent.	*H. ridibundus* per cent.	*G. haesitator* per cent.	Total Parasitism
Harlow ...	Stack bottom	15·4	4·4	20·2	40·3
Harlow ...	Stack bottom (Cage site)	4·6	18·0	11·1	33·7
Harlow ...	Stack bottom	0	42·5	7·5	50·0
Cambridge	Field plot (Cage site)	14·4	11·5	11·5	37·4

The incidence of parasitism does not appear to have changed greatly during the past 10 years for Cameron (1938) working chiefly in Essex estimated the average attack during the years 1935–36 to be 45–50 per cent.

A. quadridentatus.—The adults of this species emerged over the periods 1–23 July at Harlow and 6–27 July at Cambridge; the peak in both localities being between 10–15 July.

G. haesitator.—In both localities this species emerged over periods similar to those given for the previous parasite but there were no well defined peaks. The majority of the males emerged earlier than the females and a sex ratio of one male to two females was recorded. The latter observations are in accordance with those of Cameron.

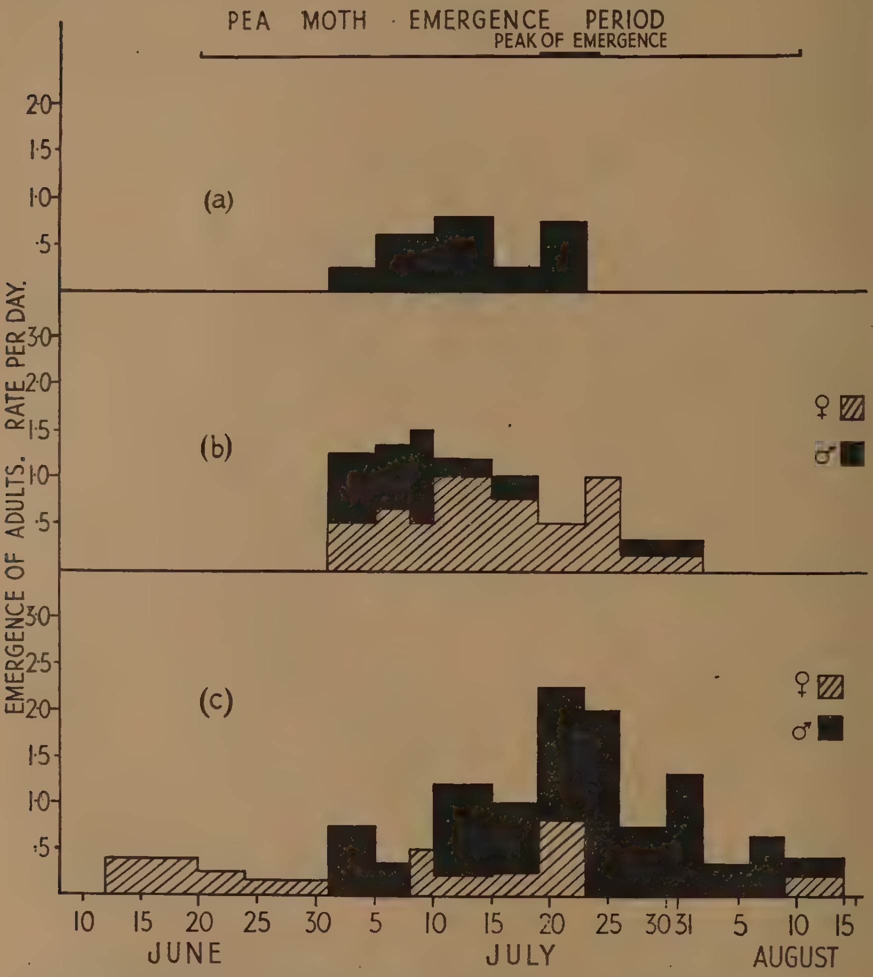


Fig. 6.—Emergence of Pea Moth parasites at Harlow, Essex, 1946. (*a*) *Ascogaster quadridentatus* (sexes not differentiated); (*b*) *Glypta haesitator* (sexes separated); (*c*) *Hemiteles ridibundus* (sexes separated).

H. ridibundus.—The emergence period of this species was much longer than that of either of the other parasites ; it began before and ended after that of its host. At Harlow the peak emergence occurred in the last week of July, coinciding with that of the Pea Moth, but at Cambridge there was no well defined peak period. There was again differentiation in emergence of the sexes but in this case the majority of the females emerged earlier than the males. The sex ratio was two males to one female.

The number of parasites from plots which had been single dug and double dug was also recorded (see p. 71). The plots were replicated, but only for one parasite, *viz. H. ridibundus*, were the data sufficiently complete to be analysed statistically. This showed that neither of the treatments caused any significant reduction in the number of adults emerging. The other parasites also emerged readily from all treatments.

The relative abundance of all three parasites in the localities sampled makes it probable that, under the conditions prevailing, methods aimed at artificially increasing their population would not be attended by any permanent success. The breeding and release of many thousands of parasites per acre would be necessary to achieve a relatively small increase in parasite attack and no method of cultural control is foreseen which would favour the parasite at the expense of the host. Under these conditions relief from this pest must be sought through other means.

Mortality in the overwintering larvae due to other causes, chiefly fungus, was usually low and for all samples taken in 1945–46 averaged 6·6 per cent.

Cultural Control.

Measures of cultural control merit full consideration, for the adoption of practices which adversely affect the Pea Moth must reduce the damage that it causes. In 1946 field experiments were carried out to determine the value of certain cultural practices which, if successful, might be considered economically useful and desirable. In addition certain varieties of peas were tested for their relative susceptibility to Pea Moth attack.

Varietal susceptibility to Pea Moth attack.

Very little is known concerning the relative susceptibility of different varieties of peas to Pea Moth attack. Two trials were carried out near Harlow, Essex, using in each case six varieties of peas. These were chosen to include early maturing types (Kelvedon Wonder and Foremost), late maturing types (Lincoln Blue and Gladstone), and two of intermediate character (Onward and Harrison's Glory). The varieties showed a wide range of haulm length from dwarf and semi-dwarf to tall. They were also chosen so that comparison could be made between several varieties commonly grown for the green pea trade (Kelvedon Wonder, Foremost and Onward) and also between two grown for the packet pea trade (Lincoln Blue and Harrison's Glory). A full account of the trials and the findings will be published later, and a summary only of the results will be given here.

In the first trial (sown 29 March), the early maturing varieties Kelvedon Wonder and Foremost came into flower well before the moths were recorded on the crops (22 June), and suffered the lowest attack. Lincoln Blue and Gladstone were the latest to come into flower and were exposed to attack over a much longer period ; these suffered the heaviest infestations. In the second trial (sown 3 May), the attack on all varieties was more uniform, with Kelvedon Wonder and Foremost more heavily attacked than in the first trial. They were exposed to attack from the beginning of flowering until harvested. The pod infestations in both trials, recorded when the varieties were ready to pick for the green pea trade, are shown in Table VII.

TABLE VII.

Varietal susceptibility to Pea Moth attack.

Variety	Percentage pod attack at " Green picking stage "			
	First Trial, sown March 29		Second Trial, sown May 3	
	Date of first flower	Pods attacked per cent.	Date of first flower	Pods attacked per cent.
Kelvedon Wonder...	29th May	6·4	24th June	19·8
Foremost	22nd May	9·8	20th June	22·6
Onward	12th June	16·3	3rd July	34·3
Lincoln Blue ...	18th June	32·8	3rd July	26·7
Harrison's Glory ...	12th June	10·8	3rd July	23·9
Gladstone	18th June	53·3	7th July	25·1

The infestation on the varieties was also found to be influenced by the amount of foliage cover provided. The cover was most dense on the plots of Onward, Lincoln Blue and Gladstone and these suffered the heaviest attacks. The importance of foliage cover in determining attack appears to be connected with the capacity of the crop to provide shelter and resting sites for the moths.

The trials showed that varieties of cultivated peas differ widely in their susceptibilities to Pea Moth attack and that the factors chiefly concerned were earliness of maturity and the degree of foliage cover. It seems probable that by selective breeding along these lines new commercial varieties of peas, less susceptible to attack than many now grown, could be produced. The trials also showed that differences of attack occur on varieties now grown for similar commercial purposes ; thus Onward was more susceptible than Kelvedon Wonder, Lincoln Blue than Harrison's Glory, whilst little difference existed between Kelvedon Wonder and Foremost.

The effect of the date of sowing on infestation.

It is widely known in horticultural circles that certain varieties of peas if sown early escape much of the severity of Pea Moth attack. From the previous section it will be seen that in order to achieve this the varieties must mature quickly (*cf.* Kelvedon Wonder and Gladstone). Peas which come into flower and mature during the flight period of the moth (approximately mid-June to the end of August), normally suffer the heaviest attacks. Crops can be grown almost free of attack if they are sown in the latter half of June or in July since they mature after the moth's flight period. The variation in attack which may occur in relation to the date of sowing is shown in Table VIII.

TABLE VIII.

Variation in attack in relation to date of sowing on the variety Thomas Laxton, 1945.

Date of Sowing	Pod attack per cent.
22nd March	1·52
21st April	18·70
22nd May	25·60
20th June	1·06
20th July	0

It will be seen that for the variety Thomas Laxton the heaviest attacks occurred on the April and May sowings. This critical period of sowing, however, will vary for different varieties and is dependent upon the time taken to reach maturity.

The effect of growing oats with peas.

It has been stated by Nicolaisen (1928), that Pea Moth attack in the field pea Maple is reduced by growing it in a mixture with oats. This was tested at Cambridge in 1946 by growing plots of Maple peas mixed with oats and plots of Maples only. There were three plots of each kind, each plot measuring 3½ yds. by 3 yds. and the layout was randomised. The pod infestations on each plot at the harvesting stage were recorded by examining the total crop and were as follows :—Maple in the mixture, 16·98 per cent., 14·85 per cent. and 17·30 per cent. ; Maple alone, 11·60 per cent., 9·55 per cent. and 10·55 per cent. The findings are, therefore, contrary to those of Nicolaisen. It appears probable that the factors leading to the heavier attack on the Maple-Oat plots were the extra shelter provided by the oats and the greater number of pods present on the pure Maple plots.

The effect of single and double digging on the mortality of the overwintering larvae.

The effect of single and double digging on the emerging population of moths was investigated in 1946. On a heavily infested stack site, undug, single dug (trench 8–10 ins.), and double dug (trench 14–16 ins.) plots were laid out in a randomised fashion ; there were three replicates of each. The plots were dug on April 4 thus giving the soil ample time to assume the same temperature gradients as the undug soil. Cages were erected on the plots on June 7 and the first moths emerged from the undug plots on June 12, from the single dug plots on June 24, and from the double dug plots on July 5. Whilst the total numbers of moths emerging from each of the treatments were 40, 51 and 14 respectively, statistical analysis failed to show any significant differences between these figures ; this was due chiefly to the small number of replicates used. Analysis, however, showed that the onset of emergence was definitely delayed by double digging.

The capacity of the overwintering larvae to penetrate the soil was further tested by placing them at various depths in drain pipes filled with soil which were then sunk to ground level. The numbers of moths emerging from larvae placed at depths of 2, 4, 6, 8 and 12 inches were not significantly different, but from 24 inches a 75 per cent. reduction in numbers occurred ; these were also later in emerging. From these results it appears that larvae which occur down to a depth of 12 ins. can produce moths within the normal emergence period, and that ploughing down to this level would not significantly reduce the moth population. Larvae which occur below 12 ins. and down to 24 ins. produce fewer moths but ploughing or digging to this depth would not be a practical proposition on most soils.

The results of the foregoing experiments suggest that in the green pea producing areas of Essex a marked reduction in Pea Moth damage would occur if the following cultural practices were adopted :—

(1) The acreage of peas grown for seed should be reduced to a minimum and if possible eliminated. This includes field peas, *e.g.* Maple, as well as the culinary varieties.

(2) The residue of crops picked green should be carted from the field immediately after picking and fed green to stock, ensiled or made into manure.

(3) Where possible early maturing varieties of green peas should be sown to be picked before the peak of Pea Moth emergence.

(4) Crops to be picked green should be kept as free from weeds as possible as these provide increased shelter for the Pea Moth ; they also result in a greater percentage of the pods remaining ungathered.

(5) Peas should be grown as far distant as possible from land on which peas had been grown the previous year. They should not be grown on a field, a part of which grew peas in the previous year.

(6) Wild vetches (*Vicia Cracca* and *Lathyrus pratensis*) should be cut early in July to prevent the pest from breeding on these host plants. They are very common on many roadsides.

(7) Forage crops containing peas should be harvested in early July before the larvae mature and leave the pods.

(8) Peas grown for seed should be followed by a crop which requires cultivation early in the next season so that volunteer plants may be destroyed.

Chemical Control.

Attempts to control the Pea Moth by chemical sprays or dusts date back nearly half a century but their use has been attended by very indifferent success. Arsenical sprays feature most widely and Fletcher (1901) obtained a reduction of 10–15 per cent. in pod attack by the use of Paris green and water. Miles (1926) in England, and Kuwayama (1937) in Japan likewise recorded decreases in attack when lead arsenate mixtures were used, and the former also found a derris extract spray gave some control. Calcium arsenate and lime dust (1 : 4) tested by Ozols (1933) reduced the number of injured seeds by 15 per cent.; whilst a Swedish worker, Schwan (1941), found that a 0·2 per cent. solution of nicotine gave a partial control.

In North America the experiments of Fluke (1921), and Hanson and Webster (1936) offered little hope of providing a method of control. More recently Baker and Perron (1944) achieved some measure of success with nicotine-oil emulsion sprays, timing the applications " to cover the period between commencement of egg laying and the disappearance of larvae into the pods".

The first recorded use of DDT against the Pea Moth was by Hey (1946), who, working with peas under cloches, showed that both dust and wet preparations of this chemical will give a considerable degree of control. His results indicated that treatment should begin before damage has commenced.

In the present investigation, work on the chemical control of the Pea Moth was begun in 1945. Preliminary experiments showed that considerable reduction in attack could be obtained by spraying the pea crop with an emulsion containing ½ per cent. DDT. These experiments were continued in 1946 and were chiefly carried out on 3 field crops of Onward peas situated near Harlow, Essex. Damage in this area from Pea Moth was known to be generally high, and to foster such infestation one experimental crop was sown on land which had carried a heavily attacked crop the previous year (4 acres experiment); and another on land which adjoined a similarly attacked crop of 1945 (3 acres experiment). The third experimental crop (11 acres), however, was situated at least half a mile from the nearest pea crop of the previous year, but was in the same area. Drilling was arranged to produce " main-crop " peas, as these are most heavily attacked, and took place on April 26 and 27 on the 4-acre and 11-acre fields, and on May 3 on the 3-acre field. The insecticide used was DDT and this was applied either as an emulsion or as a suspension. In the emulsion the DDT was dissolved in solvent naphtha and emulsified with cyclohexylamine dodecyl sulphate. The suspension was compounded with a proprietary spreader. The average particle size of the DDT in the latter was about 20μ in diameter and it contained no solid supplement as it was considered commercially undesirable to cover pods and foliage with a heavy deposit of coloured material.

The sprays were applied by a power operated machine fitted with hind and side tool bars to which the spray nozzles were attached. These were arranged so that each row of peas was sprayed by three nozzles: one nozzle, set about 9 inches above the crop sprayed downwards and the other two, set at just above crop height, were directed into either side of the row. The spray was applied at a working pressure of 300 lb. per sq. in. through fine nozzles (aperture $\frac{3}{64}$ in. in diameter). This gave a highly dispersed mist-like spray and produced on the foliage minute closely set droplets which on drying left a fine deposit of DDT. Although these droplets were most highly concentrated on the upper sides of the leaves, the undersurfaces and the stems were also partially covered with spray. There was a similar discontinuous cover on the pods and flowers. The sprays were applied either at 210 gals. or 140 gals. per acre, this difference being achieved by driving the tractor in bottom or second gear respectively. At the low rate per acre the deposit was of the same kind as with the high rate, but the droplets were more widely spaced. The wheels

TABLE IX.

Control of Pea Moth, 1946.

Summary of spraying treatments carried out on three fields near Harlow, Essex.

Treatment	Fields			
	4-acre		11-acre 1 application only— 9th July	3-acre 1 application only— 10th July
	1st application	2nd application		
(1) $\frac{3}{4}$ per cent. DDT emulsion 210 gals. per acre ...			×	
(2) $\frac{1}{2}$ per cent. DDT emulsion 210 gals. per acre ...	×		×	×
(3) $\frac{1}{2}$ per cent. DDT emulsion 210 gals. per acre ...	×	×		
(4) $\frac{1}{2}$ per cent. DDT emulsion 210 gals. per acre ...		×		
(5) $\frac{1}{2}$ per cent. DDT emulsion 140 gals. per acre ...			×	×
(6) $\frac{1}{2}$ per cent. DDT emulsion 140 gals. per acre ...	×	×		
(7) $\frac{1}{4}$ per cent. DDT emulsion 210 gals. per acre ...			×	×
(8) $\frac{1}{8}$ per cent. DDT emulsion 210 gals. per acre ...			×	×
(9) $\frac{1}{2}$ per cent. DDT suspension 210 gals. per acre ...	× ×		× ×	× ×
(10) $\frac{1}{2}$ per cent. DDT suspension 210 gals. per acre $\frac{1}{2}$ per cent. DDT emulsion 210 gals. per acre ...	× ×	×		
(11) $\frac{1}{2}$ per cent. DDT suspension 140 gals. per acre ...	× ×			
(12) Control (untreated) ...	× × ×	× × ×	× × ×	× × ×

Symbols : ×—Treated with DDT emulsion.
× ×—Treated with DDT suspension.
× × ×—Untreated (controls).

of both tractor and sprayer were adjusted to the row widths (approx. 22 ins.), and tracked each other. The plants were some 18 ins. high when first sprayed and frequently were lying across the inter-row spaces. To prevent damage by the wheels of the spraying machinery the plants were moved out of the appropriate inter-row spaces by hand labour. Very little damage was therefore caused, and where a second spray was applied the same wheelmarks were again used.

Spraying was carried out in dry, warm weather and in all cases drying was rapid. The deposit gave a slight whitish sheen to the plants and, although this was more obvious where the highest DDT rates had been used, it was never striking. None of the sprays used produced phytocidal effects.

Details of the treatments carried out in the three experimental fields are shown in Table IX. The crops on the 11-acre and on the 3-acre fields were only sprayed once as these were expected to be picked green. On the 4-acre, which by arrangement was to be harvested for seed, double applications were also tested.

Pod samples were taken from each treatment at the " green picking " stage, and where available the plots were resampled when the crop was mature and being pulled for harvesting dry (" harvesting stage "). The number of pods taken per plot varied from 200 to 333 according to the size of the plot. These were obtained by taking from each plot a random sample of complete plants from which all the pods were stripped. The pod sample could therefore be regarded as representative of the total population. The samples from the untreated fields were taken in a similar way.

4-acre Experiment.

Details of the treatments carried out on the 4-acre field experiment are shown in Table X. The first spray was applied twelve days after the crop came into flower and pods 3 ins. in length were common. Each of the eight treatments consisted of three plots, two of which measured 880 sq. yds. each and the other 660 sq. yds., making ½ acre per treatment. Peas of similar age and type also grew on an adjoining 1-acre strip which projected from the main experimental area. This remained wholly untreated. The DDT was applied at the same spray concentration in all treatments : 5 lbs. DDT (comm.) per 100 gals. of wash or ½ per cent. DDT by weight. Both emulsions and suspensions were used and the weight of DDT applied per acre was varied by changing the rate of spray application.

Pod samples were taken at the green picking and harvesting stages and from these the data given in Tables X and XI were obtained. Individual plot infestations are given to show the variability encountered, whilst the larval population from each treatment is classified according to size. The application of significance tests to the results was not possible owing to differences in size and shape of the plots. The variability in attack on plots of the same treatment was such, however, that small differences in average infestation were not taken as indicative of differences in performance of the treatments.

Consideration of the results at the green picking stage (Table X) shows that all the DDT treatments gave a considerable reduction in pod attack as compared with the control plots or with the wholly untreated corner of the field (tr. 12). Some of the treatments were much more effective than others and the more important relationships in this respect may be summarised as follows (conc. of DDT in all sprays ½ per cent. by weight).

(*a*) Two applications of emulsion each at 140 gals. per acre were quite as effective as two applications of emulsion at 210 gals. per acre (cf. trs. 6 and 3).

(*b*) Two applications of emulsion each at 210 gals. per acre were much more effective than either one application of emulsion or of suspension at the same rate (cf. trs. 3 with 2 and 9).

(*c*) One application of emulsion at 210 gals. per acre was as effective as one application of suspension at 210 gals. per acre (cf. trs. 2 and 9).

(*d*) One application of emulsion at 210 gals. per acre applied towards the beginning of the flowering period gave a much better control than a similar spray applied later and when the pods were well developed (cf. trs. 2 and 4).

TABLE X.

Four-acre Field Experiment. Sown 25th April. Summary of infestation at the "Green picking" stage. Samples taken 6th August. Eight hundred pods examined per treatment.

Treatment	Rate of application of DDT per acre at each spraying lb.	Pod attack on each plot Per cent.	Average pod attack Per cent.	Number of larvae per 1,000 pods			
				Small	Medium	Large	Total
(2) ½ per cent. DDT emulsion at 210 gals. per acre, 8th July only ...	10·5	12·7 : 8·0 : 17·5	12·1	45	41	39	125
(3) ½ per cent. DDT emulsion 210 gals. per acre, 8th and 24th July ...	10·5	9·3 : 6·7 : 4·3	6·8	16	33	23	72
(4) ½ per cent. DDT emulsion 210 gals. per acre, 24th July only ...	10·5	17·3 : 17·7 : 31·0	20·9	5	20	211	236
(6) ½ per cent. DDT emulsion 140 gals. per acre, 8th and 24th July ...	7·0	5·7 : 1·7 : 9·0	5·0	7	10	34	51
(9) ½ per cent. DDT suspension 210 gals. per acre, 8th July only ...	10·5	15·7 : 9·0 : 16·7	13·8	39	50	50	139
(10) ½ per cent. DDT suspension 210 gals. per acre, 8th July and ½ per cent DDT emulsion 210 gals. per acre, 24th July...	10·5	11·3 : 7·3 : 7·0	8·7	11	19	61	91
(11) ½ per cent. DDT suspension 140 gals. per acre, 8th July only ...	7·0	16·0 : 19·0 : 23·5	19·0	79	64	55	193
(12) Untreated :							
(*a*) Control plots	0	38·1 : 34·3 : 50·9	40·8	183	111	195	489
(*b*) Corner of field ...	0	—	57·5	190	130	190	510

TABLE XI.

Four-acre Field Experiment. Sown 25th April. Summary of infestation at "Harvesting" stage. Samples taken 20th August. Eight hundred pods per treatment.

Treatment No.	Pod attack on each plot per cent.	Average pod attack per cent.	Number of larvae per 1,000 pods			
			Small	Medium	Large	Total
(2)	26·0 : 13·3 : 18·5	19·4	24	28	149	201
(3)	7·7 : 3·0 : 5·3	5·3	3	1	49	53
(4)	20·3 : 18·3 : 27·5	21·4	4	26	196	226
(6)	4·3 : 4·7 : 8·0	5·4	3	6	45	54
(9)	22·3 : 11·3 : 24·5	18·7	30	45	119	194
(10)	10·0 : 8·0 : 7·5	8·6	4	11	73	88
(11)	22·7 : 22·7 : 27·0	23·7	34	46	171	251
(12) (*a*)	44·0 : 52·0 : 53·0	49·1	28	40	433	501
(*b*)	—	66·7	203	157	450	810

The pod infestations recorded at the harvesting stage (Table XI) show that the relationships between treatments outlined above were, in general, maintained up to the harvesting stage. The most effective treatments were again 3 and 6 and little increase in attack occurred with either between the two sampling dates.

From an examination of the larval populations from the two sets of samples further information can be obtained concerning the performances of the different spray applications. The single late application applied in treatment 4 on July 24th brought about a marked reduction of immature larvae in the green picking sampling as compared with trs. 2 or 9, or with the untreated plots (tr. 12). Furthermore, at the harvesting stage in tr. 4 there was no further recorded increase in total larvae and immature forms were again few. This situation relating to larval increase and paucity of immature forms at the harvesting stage also occurred on all treatments receiving a double application of spray. In the treatments receiving a single early application, however, the larvae at the harvesting stage showed a marked increase and included many immature forms. This indicates that the residue of the second application was almost fully effective in preventing attack for a period of four weeks after application.

The performance of the first spray application can be deduced from a comparison of the numbers and composition of the larvae in treatments receiving double applications, trs. 3, 6 and 10, with those from treatment 4 which received a single late application. At the green picking stage the immature larvae recovered from all four treatments are comparable in number and are derived, on account of their age, from the period after the second spray treatment (July 24th). The large larvae, however, originated before this second spray was applied and, in treatment 4, is some 170 more per 1,000 pods than in the average of the other treatments. This population of larvae representing, with one larva per pod, a 17 per cent. attack is, therefore, the measure of control given by the first spray during the 16 days following its application. The small number of large larvae found at the green picking stage on all plots receiving double spray applications appears to be largely of pre-spraying origin. Moths were first swept from this field eleven days before spraying and on the day of treatment eggs were common on all plots and a few of these had already hatched.

The spraying results from this field are therefore closely similar both from the point of view of pod infestation and larval population. The first DDT spray was almost fully effective in preventing attack for at least 16 days after application. During this time, however, vegetative and pod growth was occurring rapidly and produced, in the absence of further treatment, a substantial area of unprotected foliage and pods. Thus, the attack was able to continue and it gave rise to numbers of immature larvae in both the green picking and the harvesting stage samples. Where a second application was given, some 16 days after the first, this form of attack was largely prevented and little further increase in infestation occurred. Although all the double treatments gave very high degrees of control it is most probable that this would have been more complete if the first spray had been applied about three days earlier.

11-acre Experiment.

On this field six different DDT spray treatments were carried out. In each case one application only was applied, on July 9th, some eight days after the first flowers appeared on the crop. The spray was applied at 210 gals. per acre in all treatments except (5), and the concentration of DDT (comm.) in the sprays was varied between ¾ per cent. and ⅛ per cent. by weight giving rates of application of 15·75 lb. to 2·63 lb. of DDT per acre. There were three plots of each treatment and five untreated

TABLE XII.

Eleven-acre Field Experiment. Sown 26th April. Summary of infestation at "Green Picking" stage. Sprayed once on 9th July. Samples taken 30th July. Three hundred and thirty-three pods examined per plot.

Treatment	Rate of application of DDT per acre lb.	Pod attack on each plot per cent.	Average pod attack per cent.	No. of larvae per 1,000 pods			
				Small	Medium	Large	Total
(1) ¾ per cent. DDT emulsion at 210 gals. per acre ...	15·75	3·0 : 2·4 : 3·6	3·0	27	3	0	30
(2) ½ per cent. DDT emulsion at 210 gals. per acre ...	10·5	3·9 : 4·2 : 2·7	3·6	24	11	2	37
(5) ½ per cent. DDT emulsion at 140 gals. per acre ...	7·0	7·5 : 4·2 : 4·2	5·1	34	17	1	52
(7) ¼ per cent. DDT emulsion at 210 gals. per acre. ...	5·25	8·7 : 5·4 : 4·8	6·3	52	10	5	65
(8) ⅛ per cent. DDT emulsion at 210 gals. per acre ...	2·63	8·1 : 5·4 : 8·1	7·2	49	12	14	75
(9) ½ per cent. DDT suspension at 210 gals. per acre ...	10·5	6·0 : 2·1 : 5·4	4·5	21	23	2	46
(12) Control ...	0	22·8 : 23·4 : 13·8 12·6 : 25·5	19·6	111	61	37	209
Nearest untreated crops of similar age to 11-acres. 500 pods taken on each field. Sampled at the Green Picking stage :							
(*a*) Weald Bridge 4-acres Onward, sown 12th April			60·8	388	152	204	744
(*b*) Weald Bridge 3-acres Onward, sown 3rd May			33·6	184	124	72	380

plots. Plots of the same treatment were widely separated from each other and arranged at random on the experimental site. The application of significance tests to the results could not, however, be carried out as irregularities in shape of the field resulted in the plots varying both in shape and in size, the range in the latter being from 1,110 sq. yds. to 1,620 sq. yds. Furthermore, practical difficulties occurred when spraying two plots of the same treatment in one block. Reference to the pod attack (Table XII) shows that the variation from plot to plot was such that small differences in average infestation cannot definitely be attributed to the treatments but may be based on experimental error. The results given by treatments 1, 2 and 9 may therefore not appear to be significantly different. It will, however, be noted that the effectiveness of the various treatments is directly related to the dosage of DDT applied. Furthermore, this relationship is closely maintained in the 3-acre experiment, where the treatments carried out in this field were repeated. The results obtained in the 4-acre experiment also showed that the degree of control was directly related to the quantity of DDT applied. This evidence, when taken together, strongly indicates that the differences in average pod attack recorded in Table XI represent differences in effectiveness of the treatments.

The best result was given by the highest DDT rate per acre (tr. 1). It is, however, only slightly superior to that given by treatment 2; and the increase in control achieved by the additional 5 lb. of DDT per acre is in this case much less than was obtained with this weight of DDT added to a lower basic rate per acre (compare treatments 2 and 7). This indicates that, for the control of Pea Moth up to the green picking stage in this experiment, 10·5 lb. of DDT per acre applied as in tr. 2 approached the economic rate of application, being near to the optimum of maximum control at minimum insecticide outlay.

Reference to larval numbers shows that, in conformity with the level of pod attack, there were far fewer larvae in the treated samples than in the untreated. There was also a difference in the age composition of the two groups; the large larvae formed 17·5 per cent. of the total population from the untreated samples whilst from the three most effective treatments 1, 2 and 9 they represented, on an average, only 3·6 per cent. of the total population. This marked reduction in the proportion of large larvae clearly indicates that the spray deposit was giving its maximum protective effect directly after application. Its effectiveness, however, became reduced prior to sampling, allowing a number of larvae to obtain entry into the pods. The very small numbers of large larvae recovered from the most effective treatments at the green picking stage shows that the pre-spraying phase of attack must have been almost completely absent in this field. Eggs were common on the crops at the time of spraying but no hatched specimens were found. As compared with the 4-acre experiment the peas in this field were in an earlier stage of growth, being in full flower and showing only small pods, none of which was visibly swollen.

TABLE XIII.

Eleven-acre Field Experiment. Sown 26th April. Summary of infestation at "Harvesting" stage. Sprayed once on 9th July. Samples taken 16th August. Three hundred pods examined per plot.

Treatment No.	Pod attack on each plot per cent.	Average pod attack per cent.	No. of larvae per 1,000 pods			
			Small	Medium	Large*	Total
(1)	7·0 : 3·0 : 6·1	5·4	2	7	43	52
(2)	9·0 : 8·7 : 8·3	8·7	8	11	64	83
(7)	16·0 : 14·0 : 12·0	14·0	10	12	112	133
(8)	17·7 : 10·0 : 11·7	13·1	23	21	89	133
(12)	31·3 : 26·7 : 18·3 28·3	26·2	22	36	269	327

*Many of the large larvae had left the pods at this stage. Their number was assessed from the degree of seed damaged and the number of emergence holes in the pod.

Certain of the treatments were re-sampled at the time the crop was being pulled for harvesting dry (see Table XIII). The usual complete plant samples (see p. 74) could not be taken as a prolonged period of rain had caused a rotting of many of the older pods. An increase in attack was recorded in all treatments but was least on those receiving the highest dosage of DDT.

A record of the attack at the green picking stage on two fields of Onward peas, lying about ¾ mile south-east of this experimental field was also taken. These were drilled at comparable dates to the experimental field and although much smaller in size they were the two nearest untreated fields suitable for comparison. As in the case of the experimental field, peas had not been grown on these fields the previous year. The pod attack and larval numbers in a 500-pod sample from each field are shown in Table XII. Both fields show a much higher infestation than was recorded for the control plots in the experimental field. The attack in the latter was, however, almost certainly depressed by treatment of the adjoining plots, for the infestation on the untreated plots lying towards the ends of the field was almost double that on those towards the centre.

3-acre Experiment.

The treatments carried out in this 3-acre crop were the same as those on the 11-acre field except that treatment 1 was omitted. The spray was applied on

TABLE XIV.

Three-acre Field Experiment. Sown 3rd May. Summary of infestation at "Green Picking" stage. Sprayed once on 10th July. Samples taken 1st August. Six hundred pods examined per treatment.

Treatment	Rate of application of DDT per acre lb.	Pod attack on each plot per cent.	Average pod attack per cent.	No. of larvae per 1,000 pods			
				Small	Medium	Large	Total
(2) ½ per cent. DDT emulsion 210 gals. per acre ...	10·5	13·5 : 10·0 : 9·5	11·0	106	11	3	120
(5) ½ per cent. DDT emulsion 140 gals. per acre ...	7·0	14·5 : 7·5 : 13·0	11·7	88	21	8	117
(7) ¼ per cent. DDT emulsion 210 gals. per acre ...	5·25	11·5 : 10·5 : 17·5	13·2	110	23	5	138
(8) ⅛ per cent. DDT emulsion 210 gals. per acre ...	2·63	14·0 : 13·5 : 19·0	15·5	121	35	17	173
(9) ½ per cent. DDT suspension 210 gals. per acre ...	10·5	11·5 : 14·0 : 12·0	12·5	80	25	20	125
(12) Control ...	0	45·0 : 33·0 : 42·5	40·2	293	83	65	441
Nearest untreated crops of similar age to 3-acres. 500 pods taken on each field. Sampled at Green Picking stage:							
(*a*) Hobb's Cross 3½ acres Onward adjoining field on south side, sown 28th April ...			46·4	326	116	104	576
(*b*) Thrushes Bush, 2-acres Onward, 1 mile south of above field, sown 18th April ...			35·0	170	132	96	398

plots. Plots of the same treatment were widely separated from each other and arranged at random on the experimental site. The application of significance tests to the results could not, however, be carried out as irregularities in shape of the field resulted in the plots varying both in shape and in size, the range in the latter being from 1,110 sq. yds. to 1,620 sq. yds. Furthermore, practical difficulties occurred when spraying two plots of the same treatment in one block. Reference to the pod attack (Table XII) shows that the variation from plot to plot was such that small differences in average infestation cannot definitely be attributed to the treatments but may be based on experimental error. The results given by treatments 1, 2 and 9 may therefore not appear to be significantly different. It will, however, be noted that the effectiveness of the various treatments is directly related to the dosage of DDT applied. Furthermore, this relationship is closely maintained in the 3-acre experiment, where the treatments carried out in this field were repeated. The results obtained in the 4-acre experiment also showed that the degree of control was directly related to the quantity of DDT applied. This evidence, when taken together, strongly indicates that the differences in average pod attack recorded in Table XI represent differences in effectiveness of the treatments.

The best result was given by the highest DDT rate per acre (tr. 1). It is, however, only slightly superior to that given by treatment 2; and the increase in control achieved by the additional 5 lb. of DDT per acre is in this case much less than was obtained with this weight of DDT added to a lower basic rate per acre (compare treatments 2 and 7). This indicates that, for the control of Pea Moth up to the green picking stage in this experiment, 10·5 lb. of DDT per acre applied as in tr. 2 approached the economic rate of application, being near to the optimum of maximum control at minimum insecticide outlay.

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TABLE XIII.

Eleven-acre Field Experiment. Sown 26th April. Summary of infestation at "Harvesting" stage. Sprayed once on 9th July. Samples taken 16th August. Three hundred pods examined per plot.

Treatment No.	Pod attack on each plot per cent.	Average pod attack per cent.	No. of larvae per 1,000 pods			
			Small	Medium	Large*	Total
(1)	7·0 : 3·0 : 6·1	5·4	2	7	43	52
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Certain of the treatments were re-sampled at the time the crop was being pulled for harvesting dry (see Table XIII). The usual complete plant samples (see p. 74) could not be taken as a prolonged period of rain had caused a rotting of many of the older pods. An increase in attack was recorded in all treatments but was least on those receiving the highest dosage of DDT.

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3-acre Experiment.

The treatments carried out in this 3-acre crop were the same as those on the 11-acre field except that treatment 1 was omitted. The spray was applied on

TABLE XIV.

Three-acre Field Experiment. Sown 3rd May. Summary of infestation at "Green Picking" stage. Sprayed once on 10th July. Samples taken 1st August. Six hundred pods examined per treatment.

Treatment	Rate of application of DDT per acre lb.	Pod attack on each plot per cent.	Average pod attack per cent.	No. of larvae per 1,000 pods			
				Small	Medium	Large	Total
(2) ½ per cent. DDT emulsion 210 gals. per acre ...	10·5	13·5 : 10·0 : 9·5	11·0	106	11	3	120
(5) ½ per cent. DDT emulsion 140 gals. per acre ...	7·0	14·5 : 7·5 : 13·0	11·7	88	21	8	117
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(*a*) Hobb's Cross 3½ acres Onward adjoining field on south side, sown 28th April ...			46·4	326	116	104	576
(*b*) Thrushes Bush, 2-acres Onward, 1 mile south of above field, sown 18th April ...			35·0	170	132	96	398

10th July, shortly after the crop had begun flowering and when pods were both scarce and small. There were 3 plots of each treatment totalling approximately ½ acre and each plot measured 30 yds. by 24 yds. Samples taken at the green picking stage showed the infestations as above (Table XIV).

Statistical analyses of the results showed that all the DDT treatments were significantly much better than the controls but were not significantly different from each other. The effectiveness of these treatments, however, is seen to be directly related to the dosage of DDT used and in this respect closely parallels the results given on the other two field experiments. Reference to the numbers and size of the larvae shows that, as compared with the controls, all the DDT treatments brought about very marked reductions in the numbers of large larvae ; a very much lower degree of control was obtained with the small larvae. This indicates that the spray deposits were giving their most complete protection in the period following application, as was found in the 4 -and 11-acre experiments as the size of the larva is directly related to its age. This effect rapidly declined, however, and the substantial attack which occurred just prior to sampling, shown by the presence of many small larvae, was much greater than that which occurred on similar treatments in the 11-acre experiment. This loss of protection may here be attributed to two main causes : firstly to the early stage of plant growth when spraying was carried out, and secondly to the small size of the plots used. The spray was applied just after flowering had begun and when the crop was making rapid growth. Two weeks later it was estimated that the upper ⅓ of each plant, including most of the pods, had been produced since the spray was applied and therefore lacked any DDT residue. Egg laying and penetration of the pods by the larvae could consequently occur in this vital upper region of the plant almost unhindered. This situation was further aggravated by the small size of the plots, which were little more than half the area of those in the 11-acre experiment, and by a local and plentiful supply of Pea Moth. In a very short flight the moths were able to move on to treated plots from neighbouring crops and from the untreated plots. Had much larger plots been used the opportunity for mixing of the populations would have been considerably less.

In Table XIV data are also given of the infestation on two neighbouring crops of untreated Onward peas ; neither of these crops followed peas in 1945. Heavy attacks were recorded in both cases, comparable to those on the control plots on the experimental field.

Discussion.

Peas which are to be picked green can only be considered commercially satisfactory if a very low percentage of the marketable pods contain heavily damaged seed together with larvae and their remains. It is of importance, therefore, to assess the success attained in this direction in the three field experiments.

The degree of seed damage caused by a larva is directly related to its age and size. The larvae from the field samples were graded into three groups, small, medium and large, corresponding to length measurements of approximately 3·5 mm. and less, 3·6 to 6·0 mm., and 6·1 mm. and over respectively. A large larva during its development causes on an average extensive damage to three seeds whilst for a medium sized larva two seeds only are usually involved. The damage caused by a larva of the " small " group is usually restricted to one seed and is always slight, frequently being of " pin-hole " dimensions. As most of the small larvae and their damage were revealed in the pods only after careful examination, it is highly probable that both would pass unnoticed during domestic preparation of peas and would not spoil the sample for factory purposes. The presence of small larvae only will, therefore, not appreciably lower the market value of green peas. Table XV gives the percentage of the pods taken from all three experimental fields which contained at the green picking stage only large or medium larvae. This corresponds more nearly to the economic loss sustained on the various treatments than the overall figure for percentage pod attack including all sizes of larvae as given in Tables X, XII and XIV.

TABLE XV.

Commercial control of the Pea Moth at the green picking stage on the three field experiments.

Treatment	Percentage of pods attacked by medium and large larvae		
	4-acre Experiment	11-acre Experiment	3-acre Experiment
(1) ¾ per cent. DDT emulsion, 210 gals. per acre. 1 application	—	0·3	—
(2) ½ per cent. DDT emulsion, 210 gals. per acre. 1 application	7·7	1·2	1·4
(3) ½ per cent. DDT emulsion, 210 gals. per acre. 2 applications	5·3	—	—
(4) ½ per cent. DDT emulsion, 210 gals. per acre. 1 late application	20·4	—	—
(5) ½ per cent. DDT emulsion, 140 gals. per acre. 1 application	—	1·8	3·0
(6) ½ per cent. DDT emulsion, 140 gals. per acre. 2 applications	4·3	—	—
(7) ¼ per cent. DDT emulsion, 210 gals. per acre. 1 application	—	1·5	2·7
(8) ⅛ per cent. DDT emulsion, 210 gals. per acre. 1 application	—	2·5	4·6
(9) ½ per cent. DDT suspension, 210 gals. per acre. 1 application	9·9	2·5	4·5
(10) ½ per cent. DDT suspension, 210 gals. per acre 1 application and ½ per cent. DDT emulsion, 210 gals. per acre. 1 application	7·7	—	—
(11) ½ per cent. DDT suspension, 140 gals. per acre. 1 application	11·7	—	—
(12) Control. (Untreated)	28·2	9·2	13·7
Neighbouring untreated pea crops :			
(*a*)	—	29·1	18·7
(*b*)	—	17·5	20·0

Table XV shows that in the 11-acre and 3-acre experiments, where single spray applications were used, 3 per cent. or less of the pods were heavily damaged in the better treatments (2, 5 and 7). By spraying earlier on the 4-acre field a similar control would probably have been obtained as some 3–5 per cent. of the attack on all treatments on this field was due to larvae of pre-spraying origin. A 3 per cent. level of damage, as compared with some 20 per cent. on the control plots or on adjoining untreated fields, approaches the degree of control which is commercially desirable.

It will also be noted that the pod attack at the green picking stage by large and medium larvae in both the 11-acre and the 4-acre fields shows for all the DDT treatments a much lower variation between treatments than is shown by the total pod attack given in Tables X and XII. The large and medium larvae originate from eggs laid chiefly in the fortnight following spraying and as all concentrations of DDT were sufficient to give a high kill of adults and larvae for a limited time after application, a levelling out of the attack over this period could be expected. Differences in the performance of treatments became more apparent later as the treatments were wearing out. From this analysis of the effect of a single spray treatment it appears that a second application put on a fortnight after the first would give little additional reduction in the numbers of large and medium larvae found at the green picking stage. Reference to the results of the 4-acre experiment (Table X) shows that little improvement at this stage did actually result from the second spray (compare treatment 2 with 3, and 9 with 10).

The effect of different rates of spray applications can also be seen from Table XV. With a similar concentration of DDT, 140 gals. of spray per acre appears to give almost as good results as 210 gals. per acre (cf. 4-acre experiment, trs. 9 and 11, and 11-acre and 3-acre experiments, trs. 2 and 5). It does not appear to be necessary to obtain a continuous (100 per cent.) cover of the plant surface in order to secure a high level of control. As both adults and young larvae are mobile, a discontinuous cover of minute, closely spaced treated areas, such as is given by a finely dispersed spray would appear to be sufficient.

Finally, it is considered that the performance of the treatments tested was in all cases adversely affected by the conditions of the experimental layout. The relatively small size of the treated plots made it possible for moths to shelter and mature on adjoining untreated vegetation and from there to pass readily to the treated plots. Furthermore, spraying resulted in a sharp decline in the moth population over the whole of each experimental field. The attack on the control plots was consequently reduced by the spray treatments on neighbouring plots and did not usually reach the level recorded on neighbouring untreated crops (see Tables XII and XIV). A heavier attack could have been expected in view of the different cropping history of the experimental and the other fields. It is probable that all of the treatments tested would have given a higher control if used on much larger plots or on a field scale. Plots should be square, or nearly so, for experimental purposes and preferably of not less than ½ acre each.

Conclusions.

From the results of the three field experiments the following recommendations concerning the control of Pea Moth on Onward peas may be given.

On crops which are to be harvested at the green picking stage one application of ½ per cent. DDT emulsion should give a satisfactory commercial control, almost completely preventing the appearance of medium and large larvae at the picking stage. Where complete fields are to be treated a ¼ per cent. DDT emulsion will probably give an equally satisfactory control. The spray should be applied before egg hatching has commenced, which for sowings made in late April and in May would be about 7–10 days after the first flowers appear on the crop. Crops sown earlier than this should not be sprayed until about seven days after the pea moth has begun to emerge. In the 1946 season this spraying would have occurred at the beginning of the last week of June. A rate of 140 gals. per acre with adequate nozzle arrangement would appear to be sufficient.

Two applications appear to be necessary for peas which are to be harvested dry. The first should be carried out as for the green picking crop and the second should be applied about 14 days later, at the same concentration and rate per acre as for the first application. This second application is necessary to maintain a high DDT cover and thereby protect the later formed pods.

Small scale plot trials.

A number of small scale plot experiments were carried out at Cambridge to obtain information supplementary to that given by the field scale trials. In one of these, the relationship between the amount of plant surface covered by DDT deposit and the Pea Moth attack was investigated. On certain plots the crop was sprayed from above, giving a complete wetting of the upper surfaces of the plant but only a partial covering to the under surfaces and to the stems. On other plots this treatment was repeated but, in addition, spray was directed upwards from well below crop level, producing a complete wetting of the stems and under surfaces of the foliage. A $\frac{1}{4}$ per cent. DDT emulsion of the same make up as in the field trials was used in both treatments and was applied by pneumatic sprayer. The spray was applied at the rate of 240 gals. per acre for treatment of the upper surface only but where both surfaces were sprayed the rate was 50 per cent. greater. A slight run off of spray occurred in all cases. For comparison, $\frac{1}{8}$ per cent. DDT emulsion was also used as a top and under surface spray. All the DDT plots were sprayed twice, the first time shortly after the beginning of flowering and again 20 days later. The variety Thomas Laxton was used and there were three plots of each treatment with a randomised layout. Pod samples showed that all the treatments gave a similar degree of control. At the green picking stage the average attack on the treated plots was 2·8 per cent. as compared with 18·1 per cent. on the control plots whilst at the harvesting stage the attacks were 4·4 per cent. and 23·2 per cent. respectively. These results show that the partial foliage cover with DDT, was as effective in controlling Pea Moth as an almost complete cover.

The value of 'Gammexane' for the control of Pea Moth was also tested in a similar experiment on the variety Harrison's Glory. An emulsion containing 0·009 per cent. by weight of the gamma isomer of hexachlorbenzene was used. This emulsion was applied alone in one treatment and in a second as a mixed spray containing also $\frac{1}{2}$ per cent. DDT suspension. A $\frac{1}{2}$ per cent. DDT suspension was also used alone and certain plots remained untreated. Two heavy spray applications, appropriately timed, were given in each treatment. Samples taken at the harvesting stage showed that the pod infestations on the untreated and on plots receiving only 'Gammexane' were equally heavy. Similarly no improvement in control resulted from adding 'Gammexane' to the DDT suspension. The latter, however, gave a very satisfactory reduction in attack.

'Gammexane' was also tested in a dust which contained 0·39 per cent. by weight of the gamma isomer. It was applied three times at 10-day intervals beginning June 21st and at a rate of 4 cwts. per acre per application. No significant reduction in attack resulted from this treatment.

In addition to the results already outlined, the small scale experiments have also shown that Pea Moth can be controlled on the varieties Thomas Laxton and Harrison's Glory by a method similar to that worked out in the field for the variety Onward.

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References.

Baker, A. D. (1937). The Pea Moth, *Laspeyresia nigricana* Steph. on the Gaspé Coast.—Sci. Agric., **17,** p. 694.

—— & Perron, J. P. (1944). Chemical control experiments with the Pea Moth, *Laspeyresia nigricana* Steph. on the Gaspé Coast.—75th Rep. ent. Soc. Ont., p. 22.

Cameron, E. (1938). A study of the natural control of the Pea Moth, *Cydia nigricana* Steph.—Bull. ent. Res., **29,** p. 277.

Fletcher, J. A. (1901). The Pea Moth.—Rep. exp. Fms Can., 1900, p. 214.

Fluke, C. L. (1921). The Pea Moth in Wisconsin.—J. econ. Ent., **14,** p. 94.

Hanson, A. J. & Webster, D. L. (1936). The Pea Moth, *Laspeyresia nigricana.* —Bull. Wash. agric. Exp. Sta. no. 327.

Hey, G. L. (1946). DDT experiments in 1945. (Pea Moth).—Grower, **25,** p. 290.

Kuwayama, S. (1937). Notes on the Pea Moth, *Grapholitha nigricana* Steph. in Nippon. [*In Japanese.*]—Kontyû, **11,** nos. 1–2, p. 1.

Langenbuch, R. (1941). Zur Biologie des Erbsenwicklers, *Grapholitha pisana* Steph. —Arb. physiol. angew. Ent. Berl., **8,** p. 219.

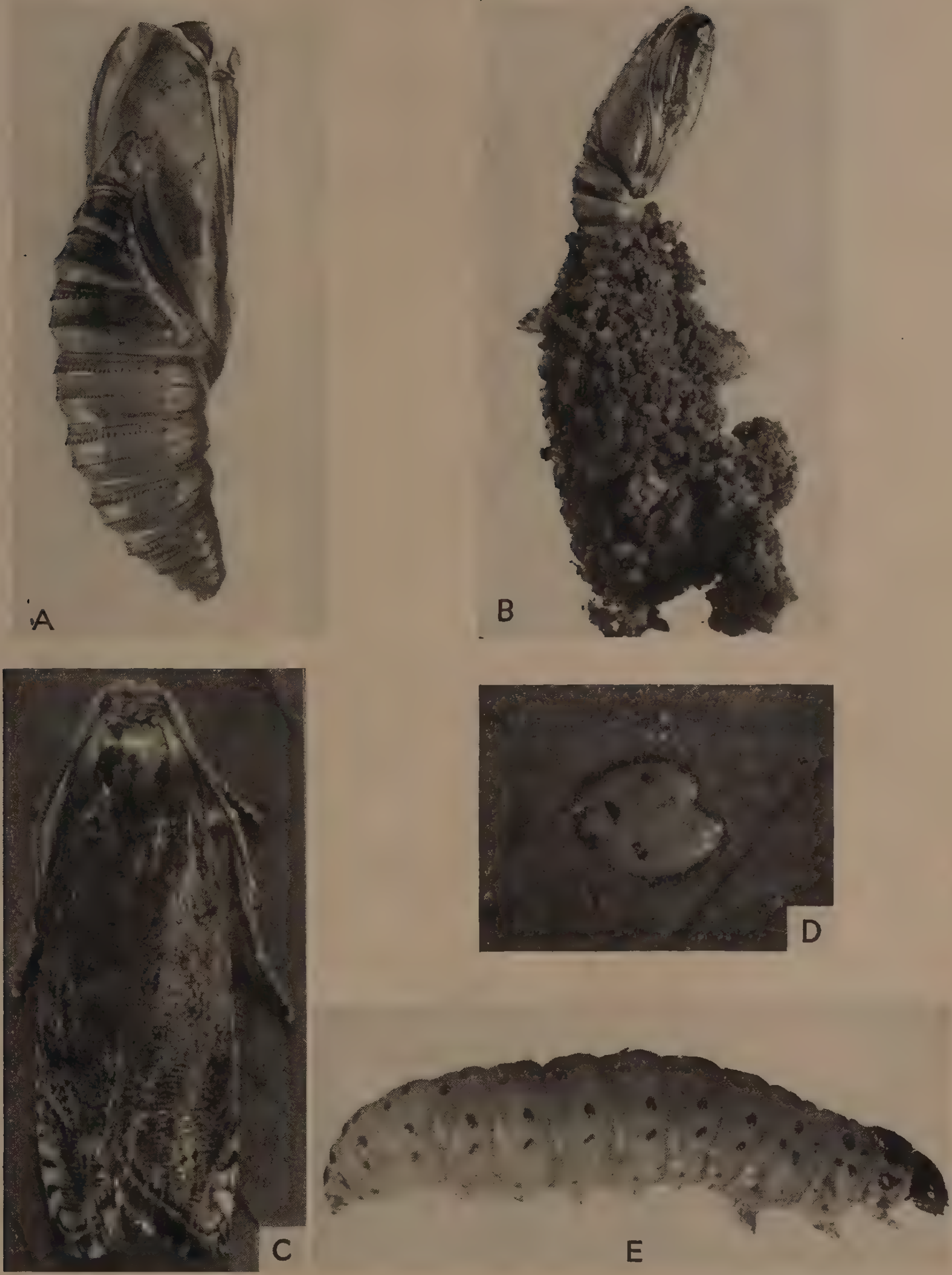
Meyrick, E. (1927). A revised Handbook of British Lepidoptera, p. 596. London.

Miles, H. W. (1926). Life-history of the Pea Moth.—Bull. Chamb. Hort., **3,** pt. 1, p. 6.

Nicolaisen, W. (1928). Der Erbsenwickler, *Grapholitha* (*Cydia, Laspeyresia*) spg., sein Schaden und seine Bekämpfung unter besonderer Berücksichtigun. der Anfälligkeit verschiedener Erbsensorten.—Kühn-Archiv, **19,** p. 196.

Ozols, E. (1933). Pea pests. [*In Lettish.*]—Lauksaimn. Menesr. no. 3, p. 130.

Schwan, B. (1941). *Cydia nigricana*—a pea pest that cannot easily be reached. [*In Swedish.*]—Växtskyddsnotiser, 1941 no. 3, p. 41.



Laspeyresia nigricana, Steph. A. Empty pupal case showing blunt cremaster and rows of dorsal spines (x 12). B. Cocoon with protruding pupal case from which adult moth has emerged (x 5). C. Adult moth in resting position. D. Two eggs at commencement of pink streak stage (x 27). E. Mature larva (x 10).

CLASSIFICATION OF THE OLD WORLD SPECIES OF THE SUBFAMILY PHLEBOTOMINAE (DIPTERA, PSYCHODIDAE).

By Oskar THEODOR.

Department of Parasitology, Hebrew University, Jerusalem.

(Plates X & XI.)

CONTENTS.

	PAGE
Introduction	85
Historical	86
Earlier attempts at classification	88
Proposed classification	92
Key to Old World ~~species~~ of the Phlebotominae ... ... subge	105
List of species of the Old World	106
References	114

Introduction.

The genus *Phlebotomus* has been much studied in recent years since the transmission of various diseases by some species has been established. It has now become a matter of importance to be able to identify species accurately in connection with the study of the epidemiology of the disease they are known or thought to transmit. It has been found that those which are closely related morphologically, often show marked biological differences and vary considerably in their rôle as transmitters of certain diseases.

A great number of species have been described in recent years, but their descriptions are widely scattered in the literature and are often not easily accessible. Many have been described repeatedly under different names. In the present paper an attempt has been made to summarise the information accumulated in the last 15 to 20 years and the classification proposed is not considered final by any means, but rather a preliminary step to a full revision of the PHLEBOTOMINAE of the Old World, which is in preparation. The author would, therefore, be grateful for any criticism of the system of classification proposed and for the correction of errors and omissions. He would also be grateful if authors who describe new species would send him their descriptions and, if possible, specimens of their new species.

Terminology.

The terminology used by most authors in the description of *Phlebotomus* is based mainly on that introduced by Newstead. This terminology has been developed in connection with the morphology of *Phlebotomus* only and has no general morphological significance. This is particularly the case in the terms used for the male hypopygium. Christophers and Barraud (1926) published a study of the development of the hypopygium of *Phlebotomus* in which the homology of the various parts was clarified and established. Their terminology has been adopted in this paper and is given below for convenience.

Male

First segment of superior clasper ... = coxite.
Second segment of superior clasper ... = style.
Intermediate appendages ... ... = parameres.
Intromittent organ ... ... = penis (consisting of penis sheet and genital filaments).
Inferior clasper ... ... = lateral lobe (of 9th tergite).
Submedian lamellae ... ... = cerci.

Female

Superior claspers ... ... = cerci.
Inferior claspers ... ... = 8th sternite. (Two halves.)
Deep appendages of Grassi ... ... = gonapophyses of 8th segment.

Wing venation.

The interpretation of the wing venation of *Phlebotomus* as given by Tonnoir (1935) has been followed. This author showed that Cu in *Phlebotomus* as in *Nemopalpus* is a convex vein which in *Phlebotomus* is very short and that M has four well-developed branches. (M_4 has been interpreted as Cu previously by most authors.)

Antennae.

Newstead's geniculated spines were shown by Feuerborn (1922) to be sense organs and given the name " ascoids ". As this name is in use for homologous structures in other groups of the Psychodids, it has been adopted here.

Buccal cavity and pharynx.

Jobling (1928) considered the terms buccal cavity and pharynx inaccurate and called the anterior pump in *Culicoides* " pharynx " and the posterior one " oesophageal pump ". Snodgrass (1935) defined the buccal cavity as the part of the stomodaeum whose dilator muscles originate on the clypeus and have their insertion anterior to the frontal ganglion, and the pharynx as the part whose dorsal dilator muscles originate on the frontal and dorsal wall of the head and are inserted posterior to the frontal ganglion. He, therefore, considered the anterior sucking apparatus of Diptera as a modification of the cibarium or buccal cavity of orthopteroid insects. According to his definition the anterior pump of the Orthorrhapha corresponds to the buccal cavity and the posterior one to the pharynx. In the higher Diptera the only pump present would also correspond to the buccal cavity and the hyoid would have to be considered as a special differentiation of the anterior part of the buccal cavity in some of the higher Diptera.

Historical.

Phlebotomus papatasi was first described by Scopoli in 1786 under the name of *Bibio papatasi*.* His description, which is practically inaccessible today, is quoted here after Grassi (1907) :

" *Bibio* (*papatasi*). Antennis, corporis, alisque pilosis. Animalculum exiguum, tenue, vix lineam longuum, pilosum. Palpi duo, articulati : articulis senis, aequalibus, oblongis, imberbibus. Rostrum obtusum, glabrum, trisetum, dificillime extricandum. Oculi reticulati, albo-punctulati, nigri. Abdomen attenuatum. Pedes longi, binis unguiculis terminati. Alae quiescentes divaricatae. Abdomen si luci appositum

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*Lavoipierre (1947, Nature, **159,** no. 4039, p. 444) records a description of a *Phlebotomus* by Philippo Bonanni in 1691. This description is of a general character and the sandfly is called " Culex minimus aculeatus Sarapico." Bonanni mentions its nocturnal and bloodsucking habits and its noiseless flight. As it seems impossible, according to Lavoipierre, to identify Bonanni's sandfly with any of the known species and as its description appeared before 1758, the date at which modern nomenclature begins, this description is of historical interest, but does not affect nomenclature.

armato oculo intueatur, rubrum apparet. Insubriae aestiva nocturnaque calamitas, aliisque Italiae Provinciis maxime infensa. Apud nos dicitur Papatasi, quod nomen triviale ritineo."

In 1840 Rondani created the genus *Phlebotomus*. His diagnosis of the genus is quoted here after his third paper (1843), in which he includes in the genus: *P. papatasi* Scop., *P. minutus* sp. n. and *P. molestus* Costa 1840. This last species is considered by Costa himself (1842) as identical with *P. papatasi*.

Genus *Hebotomus* Rondani.

Bibio Scop., *Musca* Gmel., *Cyniphes* J. Costa.

Char. gener. Antennarum articulus primus cylindricus, secundus orbicularis major, sequentes lineares 14? parum, apicis distincti, breviores. Palpi articulis duobus primis, longitudine aequales, subcylindrici, tertio breviore, quarto praecedentibus sejunctim longiore et paulo exiliore. Alarum venae decem externae; prima brevis apice secundae conjuncta, quarta et quinta basi tertiae connexae, ut septima octavae, nona decimae: sexta oriens ex vena transversa, flexaque tertiae et octavae innititur non longe a basi alarum.

Species A. Palporum articulus ultimus longitudine praecedentum conjunctim vix minor.

B. Appendices copulatoriae (marium) superiores spinulis brevissimis terminantibus, et calcaribus duobus exilibus intus praeditae.—Sp. 1. *H. papatasii* Scop.

BB. Appendices copulatoriae superiores spinis longissimis apice instructae, calcaribus interioribus indistinctis. Sp. 2. *H. minutus*, mihi.

AA. Palporum articulus ultimus longitudine vix major praecedentium sejunctim.—Sp. 3. *H. molestus* J. Costa.

Rondani in 1840 used the spelling "*Flebotomus*", in 1843 "*Hebotomus*" (obviously a misprint) and "Phloebotomidae" in 1856. Since then the spelling "*Phlebotomus*" has been generally accepted, and the name *Phlebotomus papatasi* has been included in a list of *nomina conservanda* as quoted by Handlirsch (1925). Furthermore, according to article 29 of the Rules of Nomenclature, "the original orthography of a name is to be preserved, unless an error of transcription, a *lapsus calami* or a typographical error is evident". The spelling "*Flebotomus*" is obviously an error of transcription and it therefore seems inadvisable to return to Rondani's first spelling, as proposed by Costa Lima (1932) and recently by Rapp (1944). The spelling "*Phlebotomus*" is therefore retained in this paper.

In 1907 Grassi published his classical paper, "Richerche sui Flebotomi", in which he gave a full description of the morphology and anatomy of *P. papatasi*, described for the first time the early stages and gave a review of the literature on the genus and its synonymy up to 1907. In 1908 he described a new species, *P. mascittii*, from Rome.

In 1908 Annandale and Brunetti described *P. argentipes* from India and in 1910 Annandale described five more Indian species. Brunetti (1912) gave a diagnostic table of eight Indian species and one variety.

In 1911 Newstead published his study of the Maltese sandflies, in which he described two new species, *P. perniciosus* and *P. nigerrimus*. In 1914 he made the latter a variety of *P. perniciosus*.

The first three South American species were described by Lutz and Neiva in 1912.

Subsequently, a great number of species have been described by various authors. Newstead (1911–1920) published a number of notes on species from various localities. França (1913–1925) and Parrot from 1917 onwards published a long series of papers dealing mainly with Mediterranean and African species. Sinton (1923–1933) in a series of 44 papers studied chiefly the Indian species. Adler and Theodor from 1926 onwards published a number of papers dealing mainly with Mediterranean and African species.

Further important papers are those of Nitzulescu, of Raynal and Gaschen (1935–1938) on species from Indo-China and China, a paper by Tonnoir (1935) on the Australian species and various papers by Perfiliev, Shurenkova, Chodukine and other Russian authors on the species of the U.S.S.R.

The only attempt so far to collect all the information available on the whole group is the monograph by Larrousse (1921), which deals with the 32 species and 8 varieties known at the time.

The papers mentioned above are only the most important from the systematic point of view. The literature on the group has grown lately to a great extent, since the implication of various species of *Phlebotomus* in the transmission of disease (Pappataci fever, Leishmanioses, Verruga) has made the exact identification of the transmitting insect a matter of first importance.

Earlier Attempts at Classification.

The first attempt to subdivide the genus *Phlebotomus* was made by Newstead in 1911, who divided the four Maltese species into a group with abdominal hairs recumbent and a group with abdominal hairs more or less erect. This system was also used by Sinton in 1924 for the Indian species.

In 1919 França proposed a division of the genus into two subgenera on the characters of the male genitalia, including *P. papatasi* and *P. duboscqi* Nev.-Lem. 1906 in the subgenus *Phlebotomus* and six other species from the Mediterranean and Africa in the subgenus *Newsteadia*.

In 1920 França and Parrot finding the subgenus *Newsteadia* was preoccupied, renamed it *Sergentomyia* without, however, defining a type species. They also mention a third subgenus, *Neophlebotomus*, as intermediate between the two others with the type species *P. malabaricus* Ann.

In another publication later in the same year, França defined *P. minutus* Rond. as type species for *Sergentomyia* and referred to a new subgenus *Lutzia* (emend. *Lutzomyia* França 1924) naming as type the South American species *P. longipalpis* Lutz & Neiva. The subgenus *Neophlebotomus* is not mentioned.

Larrousse (1921), quoting França and Parrot's classification of 1920, gives *P. perniciosus* as type species for *Sergentomyia*. This is inadmissible as his paper appeared after França's paper in which *P. minutus* was designated as type species for this subgenus.

In 1921 França and Parrot proposed a new system of classification, dividing the genus *Phlebotomus* into five subgenera on characters of the wing index (α/β) and the male genitalia: *Phlebotomus* s. str., *Prophlebotomus* subg. n., *Brumptomyia* subg. n., *Lutzia* França 1920 and *Sergentomyia* França 1920 (pro parte). No type species are mentioned.

Sergentomyia França and Parrot 1920 is divided into *Sergentomyia* (pro parte) ($\alpha > \beta$ *i.e.*, wing index greater than one) and *Prophlebotomus* ($\alpha \leq \beta$ *i.e.* wing index equal to or smaller than one). Type species are not specially mentioned but *P. minutus* is included under *Prophlebotomus*. This is not admissible under article 29 of the Rules of Nomenclature, which states that "if a genus (or subgenus) is divided, the type species has to remain with the restricted genus with the same name". *Prophlebotomus*, therefore, has to be considered as a synonym of *Sergentomyia* and *Sergentomyia* (pro parte) has to be renamed. *Neophlebotomus* does not appear in this classification and its type species (*P. malabaricus*) is included in *Sergentomyia* (pro parte). According to more recent knowledge, however, this species belongs to the same group as *P. minutus* and thus in the subgenus *Sergentomyia*, and *Neophlebotomus*, therefore, also becomes a synonym of *Sergentomyia*. Dyar (1929) uses the name *Neophlebotomus* for a number of American species of *Phlebotomus*. He states : " *Neophlebotomus* replaces França and Parrot's conception of *Sergentomyia* in their last work on the subject, by which subsequent authors have been guided". As *Neophlebotomus* has to be sunk as a synonym of *Sergentomyia* França 1920, this is also not permissible under the rules and both names, *Neophlebotomus* and *Prophlebotomus* cannot be used again. (Article 36 of Rules of Nomenclature.)

França and Parrot's classifications were based mainly on the structure of the male genitalia and on certain characters of the wing venation. In addition they used various measurements and indexes (" Phlebotometry "). A number of these measurements have proved valuable for the definition of species, but others introduced later by various authors have proved to be subject to wide variation within a single species and either of little or no value. In many cases the descriptions of *Phlebotomus* have been unnecessarily encumbered by the arbitrary introduction of such indexes and measurements. França and Parrot's system gave anomalous results in bringing such distantly related species as *P. sergenti* and *P. simillimus* into the same subgenus.

The main character of the wing venation on which França and Parrot's classification of 1921 was based is the wing index α/β which is either greater or smaller than one. This character is so variable in some species that some individuals of the same species could be put into different subgenera and it differs sometimes in both wings of the same individual. Furthermore, a considerable number of species (about 20) have been since described in which the wing index is constantly greater than one, but which according to all other characters, now known to be essential (male genitalia, buccal cavity, pharynx and spermathecae) belong to a group, the main character of which was, according to França and Parrot, that the wing index is smaller than one. The variability of this character has been pointed out by Magnitsky and Gutzewitsch (1929) and also by Parrot (1934) who, however, retained it in a modified form. It thus appears impossible to attribute to this character more than species value.

The size of the wing is a valuable character to estimate the size of a *Phlebotomus* as the wing is less likely to be deformed than the abdomen. The length of the wing in comparison to the body length is of value in some species (*e.g.*, *P. alexandri* and *P. sergenti*).

The body length of a *Phlebotomus* is of very limited value. There is very wide variation in size within a single species, due to the amount of food available during the larval period. In calculating the body length it is necessary to state whether the insect has been passed through KOH or not, as the abdomen is contracted in life and becomes extended considerably after treatment with KOH.

Colour is also of no great importance. There are often light and dark specimens of the same species in the same locality.

There are two formulae for the palps : the segments arranged according to their length and the relative length of the segments given in their natural order. These are very useful, but of no more than specific value.

Of the various antennal measurements, only two, the relative length of the third segment in comparison with the epipharynx $\left(\frac{A^3}{E}\right)$ and the length of the third segment as compared to the combined lengths of segment 4+5, have proved useful. The number of the ascoids (geniculated spines) on the antennal segments 3 to 15 (one or two) differs in both sexes in some groups; it either differs or not in others. In a few species (*e.g. P. squamipleuris*) there is only one ascoid per segment in both sexes throughout. Their shape and size also give some secondary characters in some species.

The morphology of the male genitalia, that is the relative dimensions of the various parts, number, shape and position of spines on the style and elsewhere, the presence of hair tufts and of various secondary appendages, are of great importance as generic and subgeneric characters.

Adler and Theodor (1926) described the structure of buccal cavity, pharynx and spermathecae of several species of *Phlebotomus* and this enabled them to identify females with certainty, which up to then could only be determined with difficulty or not at all. The discovery of these structures made it possible to establish the natural relationship of many species and thus to put classification on a new basis.

Sinton (1928) proposed a new classification, which retained Newstead's division into species with erect hairs or with recumbent hairs on the abdomen as a primary character and uses the armature in buccal cavity and pharynx and the spermathecae as secondary characters. A condensed form of this classification is as follows :—

A. *Erect haired division.*

Erect hairs on the dorsum of the abdomen. Spermathecae crenulated or striated.

Group 1. Erect hairs numerous, usually in tufts, present on most segments. Recumbent hairs scanty or absent. Buccal armature absent or rudimentary. Spermathecae and male hypopygium with specific morphology. Pharyngeal armature showing sometimes characters of secondary diagnostic importance.

Group 2. Erect hairs scanty, specially in male, often confined to first few segments. Many recumbent hairs present. Buccal armature usually well developed, spermathecae of all species similar. Male genitalia of *minutus* type.

B. *Recumbent haired division.*

Dorsal abdominal hairs, except on first segment, all markedly recumbent. Spermathecae with smooth outline. Buccal armature usually well developed and with specific morphology.

Group 3. Male genitalia very similar to those of *P. minutus.*

Group 4. Genitalia not of this type.

C. *Intermediate division.*

Only species: *P. squamipleuris.* A few erect hairs in some specimens, none in others. Spermathecae turnip shaped with rows of small spines. Buccal armature well developed. Scales on pleurae of thorax. Unilateral geniculated spines on antennae of female. Absence of these spines on 3rd antennal segments.

This classification lays too much stress on the character of the abdominal hairs. Sinton's group 2 of division A is much more closely related to the species of his division B, according to characters of buccal armature and male genitalia, than to species of division A. His creation of a separate division for *P. squamipleuris* does not appear justified for the same reason. Although this species possesses some peculiar characters, the structure of its buccal armature and male genitalia clearly shows its close relationship to the species of group 3 of this classification.

Nitzulescu (1931) proposed a classification based on the presence or absence of an armature in the buccal cavity and secondarily on the character of the spermathecae as follows :—

A. Phlebotomes a armature buccale.

1. Spermathecae crenelées.

    Subgenus *Sintonius*. Type *P. hospitii.*

2. Spermathecae non crenelées.

    Subgenus *Brumptius*. Type *P. minutus.*

B. Phlebotomes sans armature buccale.

3. Spermathecae crenelées a col plus au moins long, pharynx comme chez *P. major.*

    Subgenus *Larroussius*. Type *P. major.*

4. Spermathecae crenelées a col court.

    Subgenus *Phlebotomus*. Type *P. papatasi.*

5. Spermathecae lisses.

    Subgenus *Adlerius*. Type *P. chinensis.*

This classification is an advance on previous attempts, but it is still artificial and it does not sufficiently take into account the structures of the pharynx and particularly of the male genitalia, which furnish some of the most important characters. It also gives anomalous results as it includes *P. papatasi*, *P. sergenti* and *P. argentipes* in the same subgenus, species which differ markedly in the male genitalia and pharyngeal armature.

Theodor (1932a) proposed a tentative system of classification, without, however, naming the various groups. He erected the subfamily PHLEBOTOMINAE to contain the blood-sucking forms of the former genus *Phlebotomus*. The subfamily was divided into three tribes, which were subdivided into genera and subgenera. As the principles of division are the same as those used in the present paper, no further comment is necessary at this point.

Raynal (1935) also gave a tentative classification without defining status and naming the various groups, based mainly on the structure of the spermathecae and secondarily on the male genitalia and armature of buccal cavity and pharynx. The same criticism applies to this classification as to that of Nitzulescu. It is artificial and brings such distantly related species as *P. papatasi* and *P. argentipes* into the same group. The character of the spermathecae, although certainly of more than specific value, is not fundamental enough to serve as the main character for division.

Parrot (1934) proposed a division of the genus *Phlebotomus* into two subgenera as follows :—

> Subgenus *Phlebotomus* : bouche inerme ; indice alaire supérieur ou au moins égal a l'unité ; poils des tergites abdominaux uniformément dressés ;—parasites habituels des animaux a sang chaud. Type : *P. papatasii* (Scop., 1786).
>
> Subgenus *Prophlebotomus* : França et Parrot 1921 : bouche armée de dents ; indice alaire généralement inférieur ou a peine égal a l'unité ; poils des tergites abdominaux uniformément ou presque uniformément couchés ;—parasites habituels des animaux a sang froid. Type : *P. minutus* Rondani 1843.

As shown above, the name *Prophlebotomus* has to be sunk as a synonym of *Sergentomyia* and the wing index α/β is too variable to be used as a subgeneric character and is only of value for the distinction of species.

Kirk and Lewis (1946) recognised three subgenera of the genus *Phlebotomus* in Africa as follows :—

Phlebotomus s. str. Species with numerous erect hairs on the abdominal tergites, usually occurring in tufts on the distal ends of the segments ; buccal armature and pigmented area absent or rudimentary ; the heavily chitinised parts of the spermathecae segmented in their entire length ; spermathecal chitinisations and male hypopygium usually having specific characters.

Sintonius Nitzulescu 1931. Species with scanty erect hairs on the dorsal aspects of segments II to VI, such hairs often confined to segments II and III, especially in the males ; dorsal recumbent hairs usually numerous ; buccal armature and pigmented area usually well developed and showing specific morphology ; spermathecae crenulated ; male hypopygium usually of the *minutus* type, with pointed intromittent organ and hooked intermediate appendage.

Prophlebotomus França and Parrot 1921. Species having dorsal abdominal hairs on segments II to VI all recumbent ; buccal armature and pigmented area usually well developed and having specific morphology ; the body of the spermathecal chitinisations usually with a smooth outline, and any traces of annulation, if present, confined to the distal end.

This is a modification of Parrot's classification of 1934. *Sintonius* differs from *Prophlebotomus* only by the presence of scanty erect hairs on the abdomen and in the form of the spermathecae, while there is no difference in the buccal armature and in the male genitalia. There are, however, species with partly segmented spermathecae in the subgenus *Prophlebotomus*, while in several species of *Sintonius* the spermathecae are also incompletely segmented. As pointed out by Kirk and Lewis, the males of both these subgenera are difficult to distinguish. Although *Sintonius* is a well defined group, it does therefore not seem justified to grant it equal systematic rank with *Phlebotomus* and *Prophlebotomus*, of which latter group it clearly forms a subdivision.

The authors, furthermore, based the definition of their subgenera only on Ethiopian species. Some of their definitions are, therefore, too narrow and would not allow the inclusion of species of these groups from other areas.

Proposed Classification.

Since the publication of the various attempts of classification discussed above (except the last one), a great number of species have been described. There are now 127 species and 34 varieties known from the Old World and about 150 from America. A study of the original descriptions of nearly all the Old World species, and of specimens of about a hundred species and varieties, has made it possible to determine more correctly the natural relationship of the various species and the relative importance of the various characters used in classification.

Old World sandflies fall naturally into two main groups, *Phlebotomus*, which contains the species without an armature in the buccal cavity, and *Sergentomyia* of the proposed classification, which contains the species with a well-developed buccal armature.

The species of the genus *Phlebotomus* have either no trace of teeth in the buccal cavity or only a few isolated small teeth, which never form a definite armature. The hairs on the dorsum of the abdomen are mostly erect and stand in tufts, and the spermathecae are either completely or incompletely segmented. The pharyngeal armature in this genus gives valuable characters for the definition of subgenera. In most species these structures are very similar in both sexes, but in a few there is such a strong reduction in the male that they appear different (*P. sergenti*, *P. chinensis*). There is much more variation in the form of the male genitalia than in *Sergentomyia*, particularly in the form of the style, the number of the spines on the style and their position and there are various secondary appendages and hair tufts on the parameres and coxite. Some of the structures mentioned apparently have more than species value and the subgenera given below are based on the combined characters of the male genitalia, spermathecae and pharyngeal armature.

The genus *Phlebotomus* contains nearly all the species that feed on man and mammals and all those that are implicated in the transmission of disease.

In the genus *Sergentomyia*, the group provided with a buccal armature, the structure of the male genitalia is much more uniform than in the genus *Phlebotomus*. There are usually four long spines in the style with a small seta on inner aspect also present. There are only two aberrant species known, one with two and one with seven spines on the style. (This last species is probably an abnormality.) The parameres and the lateral lobes of the 9th tergite are unarmed and simple. The structure of the male genitalia is remarkably constant throughout the whole group and variation occurs mainly in the position of the four spines on the style, which are all terminal in some species; in a number of others two of these are either subterminal or situated in the middle of the segment and in the position of the fifth seta. The form of the penis and the paramere also varies.

The main variation in this genus is in the number, shape and arrangement of the teeth and in the form of the pigmented area in the buccal cavity and in the armature of teeth in the pharynx. This is particularly evident in the *africana* group, where a number of varieties have been described mainly on differences in the number of teeth and form of the pigmented area in the buccal cavity, and in the *fallax* group. The majority of the species of these groups occur in a continuous geographical area—the *fallax* group in the Mediterranean and Tropical Africa with only two species in India, while the *africana* group may be divided into the African-Mediterranean and East-Asiatic sections.

In many species of the genus *Sergentomyia* there is a marked sexual dimorphism in the development of the armature in the buccal cavity and pharynx, which are much more weakly developed in the male, but as a rule resemble that of the female.

The spermathecae in this genus vary in form to a certain extent. They are mostly smooth or crinkled tubes or capsules in the species with only recumbent hairs on the abdomen. There are, however, a number of aberrant forms, such as *P. squamipleuris*, in which they are round capsules covered with transverse rows of small spines, and others in which the spermathecae show crenulation of incomplete segmentation.

In most species of the subgenus *Sintonius* of the proposed classification (group 2 of division A of Sinton) the spermathecae are segmented, but in a few they are incompletely segmented. There are also scanty erect hairs on the abdomen and this subgenus may be considered as a transition to the genus *Phlebotomus*.

The form of the spermathecae is constant in the *fallax* and *africana* group. Some species occurring in the same geographical area have similar spermathecae and differ mainly in the buccal cavity and pharynx (*S. sogdiana*, *S. grekovi* and *S. sumbarica* in Turkestan and the group of Philippine species).

As already stated the wing index α/β varies widely in this genus (from 0·3 to 4·5).

Only a few species of this genus are known to bite man (*S. schwetzi*, *S. africana* and *S. babu*) and some are known to feed on birds and reptiles.

Two quite aberrant species have been described from caves in the Belgian Congo and have been shown by Wanson (1942) to feed on bats. One of these (*P. gigas*) has no armature in the buccal cavity and the other (*S. mirabilis*) has a well developed one. Like most bat parasites and many cave dwelling animals, both these species are so modified that it is difficult to place them in any of the subgenera given below.

They have the greatly elongated antennae and legs of some cave insects and the eyes are markedly reduced in *P. gigas*. They are provisionally placed in separate subgenera of the two genera, but it might eventually prove more convenient to separate them completely from *Phlebotomus* and *Sergentomyia*.

In defining the subgenera of the proposed classification, an attempt has been made to make these as natural as possible. It has, however, been found impossible to give some characters equal value in both *Phlebotomus* and *Sergentomyia* as differentiation has apparently developed along different lines in the two genera. In *Phlebotomus* there is marked variation mainly in the male genitalia and the pharyngeal armature and to a smaller degree in the spermathecae, while in *Sergentomyia* the male genitalia are relatively uniform throughout the genus and differentiation has occurred mainly in the buccal armature and the spermathecae. Thus, the subgenera of the genus *Phlebotomus* are based primarily on the structure of the male genitalia, in combination as far as possible, with pharyngeal armature and spermathecae. Two of the subgenera however (*Larroussius* and *Adlerius*) have very similar genitalia, but the differences in pharynx and spermathecae are so marked, that their separation appears justified.

Similarly, the subgenera *Paraphlebotomus* and *Synphlebotomus* show a resemblance in the male genitalia, but differ in the number of spines on the style and the pharyngeal armature. A third group of two closely related subgenera are *Euphlebotomus* and *Anaphlebotomus* which differ mainly in the number of spines on the style. There are thus six subgenera which are well defined by all three essential characters, while three other subgenera are each more or less closely related to one of the six well defined subgenera.

In the genus *Sergentomyia*, the subgenus *Sintonius*, which is a rather homogeneous group, is based primarily on the segmented spermathecae and secondarily on the presence of some erect hairs on the abdomen.

The other species of the genus (except *P. mirabilis*, which is placed in a separate subgenus) have all been retained in the subgenus *Sergentomyia*, which has been divided into groups, as the distinguishing characters do not seem to be sufficiently well marked to justify the creation of further subgenera.

A few species show such marked individual characters, that it is difficult to fit them into groups. In order to avoid the creation of too many groups containing a single species, they have been placed in a list following the groups without attempting to define their exact position for the moment. A number of other species are either insufficiently described, doubtful or known by one sex only, so that it is impossible to place them into groups as long as the other sex is not known.

The American species are not well enough known for a definite classification. The majority of the species which have been fully described have a buccal armature of a certain type, which differs from that of the Old World species and a small number of species has no armature. It seems advisable to keep both these groups in separate genera, which will eventually have to be subdivided. In the present classification the species with an armature in the buccal cavity are placed into the genus *Lutzomyia* França. More study is needed to determine whether the American species without a buccal armature can be put into the same group as the Old World species without an armature (the genus *Phlebotomus*) or will have to be kept separate. For the present, they are provisionally put into the genus *Brumptomyia* França and Parrot.

As pointed out by Tonnoir, " Phlebotomists " have often neglected to take into consideration that " phlebotomes " are Diptera and that a terminology for this group already exists and further, that the family PSYCHODIDAE contains a number of forms that are either more or less closely related to *Phlebotomus*. Tonnoir, therefore, rejects the tentative proposal of Adler and Theodor (1929) to create a separate family for *Phlebotomus*. The definition of the subfamily PHLEBOTOMINAE, however, to contain the former genus *Phlebotomus* only, seems justified not only on morphological grounds, but also because the habit of bloodsucking has developed in this group only and with it corresponding morphological changes in the mouthparts. There is only one doubtful record of bloodsucking of a member of the subfamily PSYCHODINAE (*Pericoma townsvillensis* Taylor 1915). No description of the mouthparts of this species is given and as *Phlebotomus queenslandi* Hill has been described later from the same locality, the possibility must be considered, that the biting insects were in fact *Phlebotomus* and not the *Pericoma* described by Taylor.

Tonnoir (1933) described the peculiar new genus *Horaiella* which possesses well-developed, toothed mandibles. He states : " The maxillae are not more developed than in the Psychodinae ; the lack of development of the maxillae casts some doubt on the possibility of this species being a blood sucker. (In a letter Dr. Hora says that while collecting in the localities where this species is abundant he has never been bitten by it.) Fully developed mandibles associated with reduced maxillae are found in females of some Blepharoceridae and Ceratopogonidae which are not haematophagous."

There is, therefore, no established record of blood-sucking in the family PSYCHODIDAE except by members of the subfamily PHLEBOTOMINAE.

FAMILY PSYCHODIDAE.

Small (1 to 4 mm.), hairy, moth-like Nematocera with arched thorax without V-suture and depressed head. Ocelli absent. Antennae 12 to 30 segmented. Palps with 4 to 5 segments. Proboscis short. Wings covered with hairs, veins nearly all longitudinal. Cross veins indistinct. Sc short. Rs with 3 or 4 branches. M with 4 branches. Cu either normal or much reduced. A much reduced or absent. Male genitalia prominent, consisting of 2 or 3 appendages, inverted in some groups. Some species termitophilous.

Larvae eucephalous, generally amphipneustic, sometimes very specialised, aquatic or living in moist soil. Pupae free, mobile or inactive, with thoracic respiratory appendages or not.

The family PSYCHODIDAE has been subdivided by Alexander (1929) into four subfamilies on characters of the wing venation and his classification has been accepted by Tonnoir and Edwards. This classification has been adopted here with slight modifications.

Tonnoir (1933) hesitated to place the genus *Horaiella* into the subfamily TRICHOMYIINAE although it has a 3-branched radius. In *Horaiella* R 2+3 is a simple vein, while in *Trichomyia* and *Sycorax* it is branched. He was also doubtful whether it could be placed in the subfamily PHLEBOTOMINAE although the presence of a well-developed mandible and other characters show a close relationship to *Phlebotomus*. The question of the systematic position of *Horaiella* will have to be left open for the present.

SUBFAMILY PSYCHODINAE.

Eyes kidney-shaped. Sc short. Rs with four branches. R4 originating near wing base. Cu long, parallel to M4. Cell M3 and M4 of approximately equal width. Palps four segmented. Mouth-parts not formed for blood-sucking.

Genera : *Psychoda* Latr., *Pericoma* Walk., *Clytocerus* Eat., *Telmatoscopus* Eat., *Termitadelphos* Holmgr.

SUBFAMILY TRICHOMYIINAE.

Eyes round. Rs with three branches. Cu long or short. Mouth-parts not formed for blood-sucking. Palps four segmented.

Genera : *Trichomyia* Halid., *Sycorax* Halid., *Termitodipteron* Holmgr.

SUBFAMILY BRUCHOMYIINAE.

Eyes round. Rs four branched dichotomously. R_2+_3 and R_4+_5 present. Cu short or moderately long. A present. Cell M_4 much wider than cell M_3 at wing margin. Palps five segmented. Mouth-parts not formed for blood-sucking.

Genera : *Nemopalpus* Macq., *Bruchomyia* Alex.

SUBFAMILY PHLEBOTOMINAE.

Eyes round. Sc short. Rs four branched, pectinated. R_4 originating in middle of wing, R_5 originating proximal to R_4 from Rs near wing base. Cu very short, A absent. Palps with five, antennae with 16 segments. Mouth-parts adapted for blood-sucking. Specialised armatures of teeth in buccal cavity and pharynx.

(The presence or absence of these armatures in the other subfamilies has not yet been fully established. Although their exact function is not clear, it seems probable that they have developed in connection with the habit of blood-sucking. They are absent in several species of *Psychoda* examined, in *Nemopalpus capensis* and in *Trichomyia urbica*.)

Genus Phlebotomus Rondani 1843.

Type : *Phlebotomus papatasi* Scopoli 1786.

Hairs on abdominal tergites all erect. Armature in buccal cavity absent or rudimentary. Spermathecae segmented or incompletely segmented. Male genitalia : Style as a rule with four or five spines, three in one subgenus.

1. Subgenus *Phlebotomus*. Type : *P. papatasi* Scopoli.

Male genitalia long. Style long and slender with three short spatulate spines at tip and two nearer middle of segment. Parameres with two long secondary appendages. Penis short and conical. Lateral lobes with short spines at tip, shorter than coxite. Small tubercle with hairs near base of coxite (fig. 1). Pharynx with armature appearing as network of lines (Pl. X, fig. 1). Spermathecae segmented with terminal knob (fig. 1).

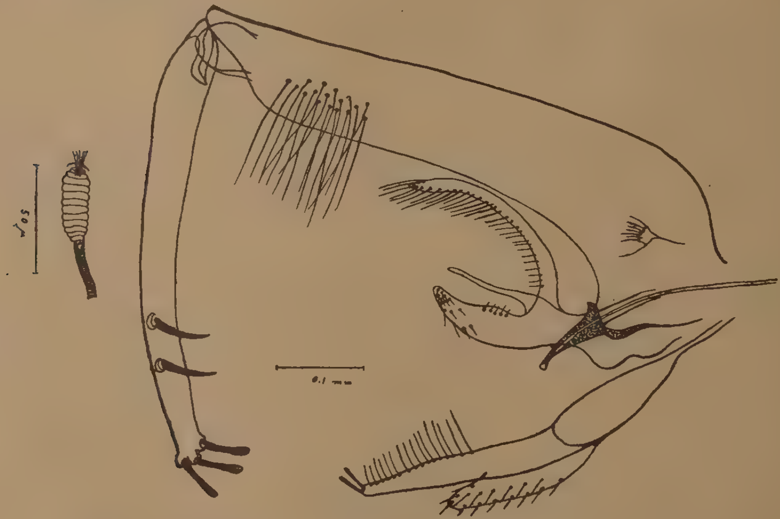

Fig. 1.—*Phlebotomus* (*P.*) *papatasi*. Male genitalia and spermatheca.

2. Subgenus *Paraphlebotomus* **nov.** Type : *P. sergenti* Parrot.

Male genitalia short. Style short with four long spines of which two are terminal or subterminal and two nearer base of segment. Process with brush of long hairs near base of coxite (Pl. XI, fig. 6). Parameres with flat elliptical upper surface. Penis short with terminal hook. Lateral lobes unarmed, longer than coxite (fig. 2). Pharynx with armature of coarse wedge-shaped teeth (Pl. X, fig. 2). Spermathecae segmented with terminal segment enlarged (fig. 2).

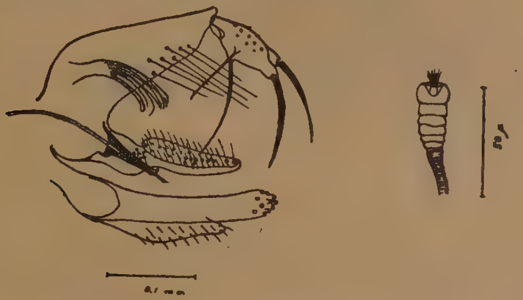

Fig. 2.—*Phlebotomus* (*Paraphlebotomus*) *sergenti*. Male genitalia and spermatheca.

3. Subgenus *Synphlebotomus* **nov.** Type : *P. martini* Parrot.

Style short with five long spines of which two are terminal and three nearer middle of segment. Process near base of coxite and parameres as in *Paraphlebotomus*. Penis short and conical (fig. 3*a*). Pharynx with armature appearing as irregular lines of medium and small teeth (Pl. X, fig. 3). Spermathecae segmented (fig. 3*b*).

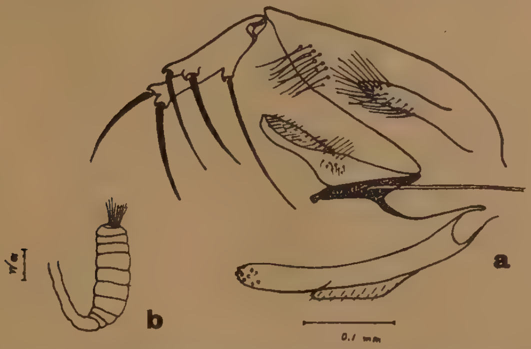

Fig. 3.—(a) *Phlebotomus* (*Synphlebotomus*) *katangensis*. Male genitalia. (b) *Phlebotomus* (*S.*) *martini*. Spermatheca (after Parrot).

4. Subgenus *Larroussius* Nitzulescu 1931. Type : *P. major* Annandale.

Style short with five long spines of which two are terminal and three nearer middle of segment. Parameres simple with club-shaped apex. Penis long, of varying form (fig. 4). Pharynx with armature of numerous small point-like teeth (Pl. X, fig. 4). Spermathecae segmented with long terminal process (fig. 4).

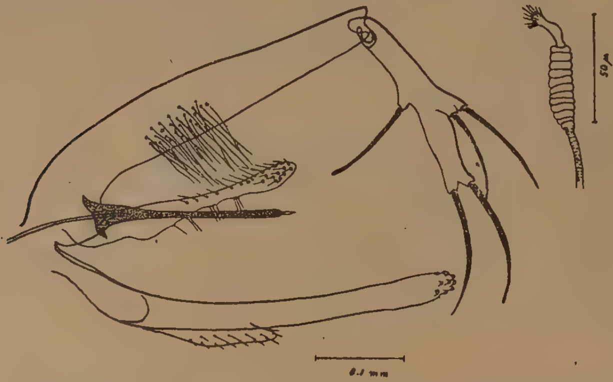

Fig. 4.—*Phlebotomus* (*Larroussius*) *major*. Male genitalia and spermatheca.

5. Subgenus *Adlerius* Nitzulescu 1931. Type : *P. chinensis* Newstead.

Male genitalia similar to those of *Larroussius* (fig. 5). Pharynx with triangular or rounded armature of medium sized teeth (Pl. X, fig. 5). Spermathecae incompletely segmented (fig. 5).

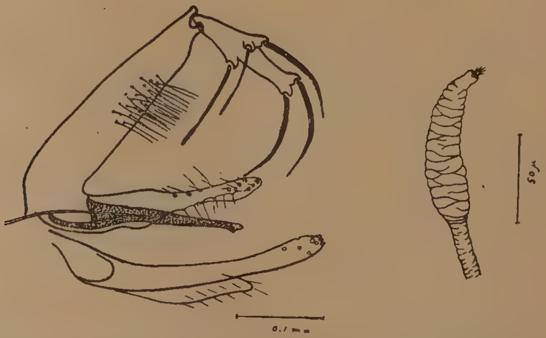

Fig. 5.—*Phlebotomus* (*Adlerius*) *chinensis* var. *simici*. Male genitalia and spermatheca.

6. Subgenus *Euphlebotomus* **nov.** Type : *P. argentipes* Annandale & Brunetti.

Style with five long spines, similar to that of *Larroussius*. Parameres trilobed, with or without accessory spine. Penis short and conical (fig. 6). Pharynx with median armature of small teeth and posterior parallel ridges (Pl. X, fig. 6). Spermathecae segmented or striated with apical segment defined and enlarged (fig. 6).

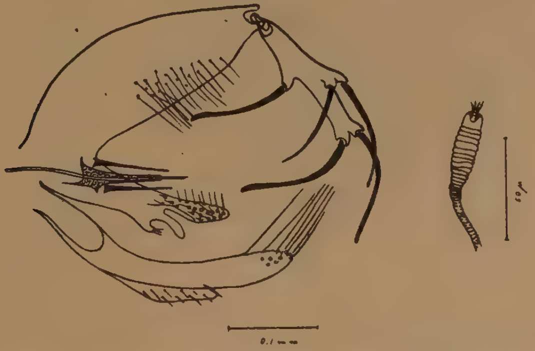

Fig. 6.—*Phlebotomus* (*Euphlebotomus*) *argentipes*. Male genitalia and spermatheca.

7. Subgenus *Anaphlebotomus* **nov.** Type : *P. stantoni* Newstead.

Style with four long spines of which one is terminal, one subterminal and two nearer middle of segment. Parameres with two or three lobes, with or without accessory spine. Penis short and conical (fig. 7). Pharynx similar to that of *Euphlebotomus* (Pl. XI, fig. 1). Spermathecae segmented, apical segment not enlarged (fig. 7).

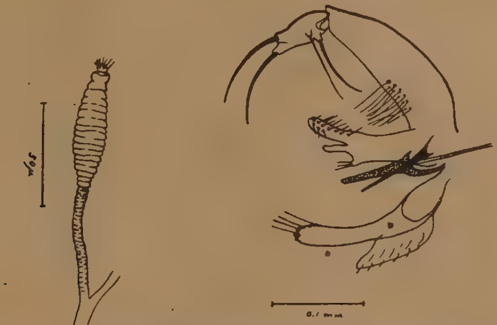

Fig. 7.—*Phlebotomus* (*Anaphlebotomus*) *stantoni*. Male genitalia and spermatheca (after Raynal).

8. Subgenus *Australophlebotomus* **nov.** Type : *P. brevifilis* Tonnoir.

Male genitalia very short. Style with three long spines of which two are terminal and one near middle of segment. Parameres simple. Penis sheet rudimentary, genital filaments very short and thick (fig. 8). Pharynx with armature of small scale like teeth. Spermathecae incompletely segmented (fig. 8).

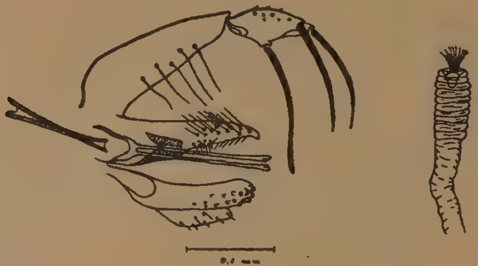

Fig. 8.—*Phlebotomus* (*Australophlebotomus*) *brevifilis*. Male genitalia and spermatheca.

9. Subgenus *Spelaeophlebotomus* **nov.** Type : *P. gigas* Parrot & Schwetz.

Eyes reduced. Legs, antennae and male genitalia much elongated. Style with four spines of which one is terminal and three others at various intervals along the segment and a small seta (fig. 9). Pharynx with a few scale like-teeth. Spermathecae wide, smooth ducts ending in narrow more strongly chitinised tubes (fig. 9). (Cave form.)

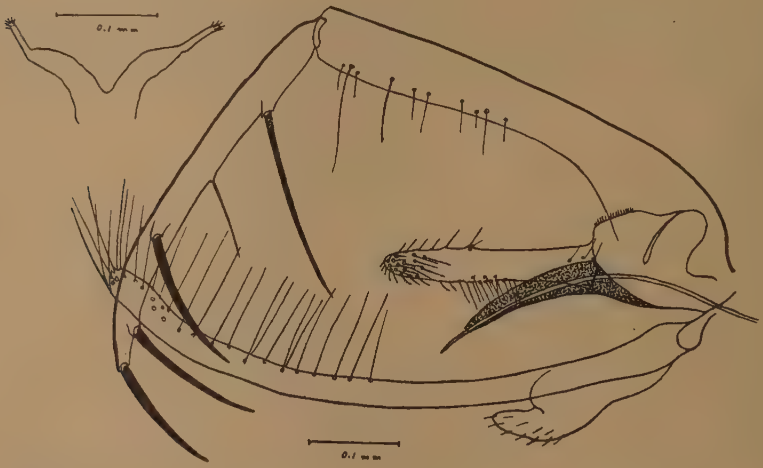

Fig. 9.—*Phlebotomus* (*Spelaeophlebotomus*) *gigas*. (a) Male genitalia. (b) Spermatheca (after Parrot).

Genus Sergentomyia França & Parrot 1920.

Type : *Sergentomyia minuta* (*Phlebotomus minutus*) Rondani 1843.

Hairs on abdominal tergites recumbent in most species. A few erect hairs on abdominal tergites in one subgenus. Well developed armature in buccal cavity.

Spermathecae tubular, smooth capsules or segmented. Male genitalia : style with four long spines and a small seta on inner aspect also present (fig. 10*a*). (Two long spines and a small seta in one subgenus.)

1. Subgenus *Sergentomyia* França & Parrot 1920. Type. *S. minuta* Rondani.

Abdominal hairs all recumbent. Style with four long spines and a small seta. Spermathecae of various forms, smooth or crinkled tubes or smooth capsules with or without spines, but not segmented.

Group minuta.

Buccal armature comb-like, consisting of 50 to 80 narrow parallel teeth. Pharynx lampglass shaped with a few teeth posteriorly (Pl. XI, fig. 4). Spermathecae tubular (fig. 13*a*). Style with four terminal spines. Parameres with blunt tip. Penis blunt but tapering (fig. 10*a*).

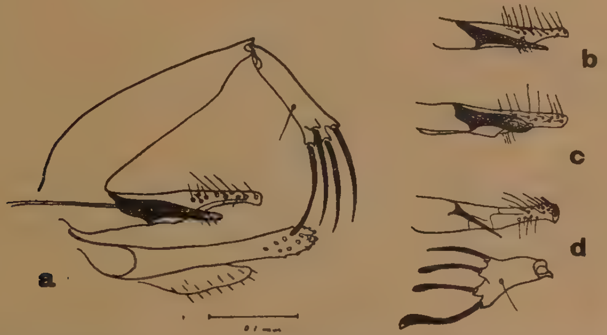

Fig. 10.—(a) *Sergentomyia* (*S.*) *minuta*. Male genitalia ; (b) *S.* (*S.*) *africana*. Paramere and penis ; (c) *S.* (*S.*) *theodori*. Paramere and penis ; (d) *S.* (*S.*) *nicnic*. Paramere, penis and style.

Group fallax.

Section a. Buccal armature consisting of pointed teeth standing on an arc (fig. 12 ; Pl. XI, fig. 5). Pharynx sometimes very wide posteriorly, always with an armature of numerous small teeth (Pl. XI, fig. 2). Spermathecae tubular. Style with four terminal spines of which two may be subterminal. Parameres with blunt tip. Penis blunt, with wide apex (fig. 10*c*).

Section b. Same, but pharynx unarmed.

Group africana.

Buccal armature consisting of varying number of parallel teeth with short or long points (fig. 12*b*). Pharynx lampglass shaped, in most species with a narmature of pointed teeth. In some species armature reduced to ridges or scales. Spermathecae elliptical or round capsules (fig. 13*b*). Style with four terminal spines of which two may be subterminal. Parameres hooked or pointed, penis pointed (fig. 10*b*).

Group zeylanica.

Section a. Buccal armature consisting of a row of long pointed teeth and one or more rows of small vertical teeth in some species (fig. 12*c*). Pharynx with ridges only. Spermathecae tubular, sometimes crenulated or weakly chitinised cylindrical capsules. Style with four long spines, two terminal and two in middle of segment. Parameres hooked. Penis pointed (fig. 11).

Section b. Same, but style with four spines either all terminal or two terminal and two subterminal.

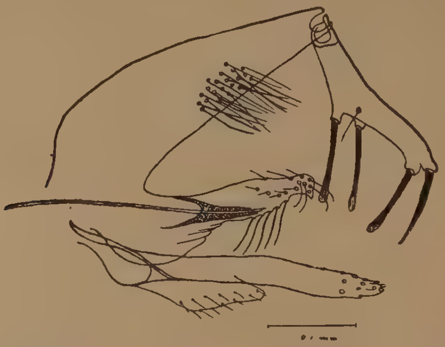

Fig. 11.—*Sergentomyia* (*S.*) *zeylanica.* Male genitalia and spermatheca.

Group squamipleuris.

Buccal armature consisting of comb-like row of parallel teeth with short points; armature convex posteriorly (Pl. XI, fig. 3). Pharynx with armature of varying number of short or longer teeth. Spermathecae round capsules with transverse rows of short spines (fig. 13*d*). Style with four terminal spines. Parameres hooked, penis pointed.

Group nicnic.

Buccal armature consisting of several irregular rows of minute teeth (fig. 12*d*). Pharynx unarmed. Spermathecae weakly chitinised, smooth elliptical or cylindrical capsules. Style with four spines, either all terminal or two terminal and two subterminal. Parameres hooked, penis pointed (fig. 10*d*).

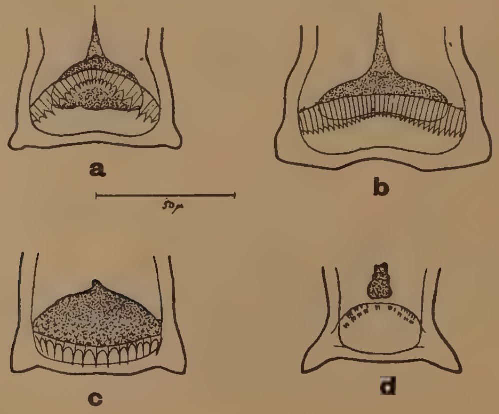

Fig. 12.—Buccal cavity of female of: (a) *Sergentomyia* (*S.*) *theodori*; (b) *S.* (*S.*) *africana*; (c) *S.* (*S.*) *zeylanica*; (d) *S.* (*S.*) *nicnic.*

Aberrant Species.

Insufficiently known or described Species.

2. Subgenus *Sintonius* Nitzulescu 1931. Type : *S. hospitii* Sinton.

Scanty erect hairs on abdominal tergites. Style with four spines, either all terminal or two terminal and two subterminal. Parameres hooked, penis pointed. Spermathecae segmented or crenulated. Buccal cavity of varying form, pharynx lampglass shaped with a few teeth posteriorly or with ridges only (fig. 14).

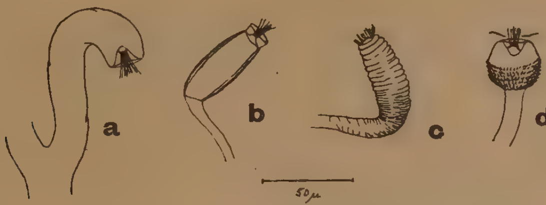

Fig. 13.—Spermatheca of : (a) *Sergentomyia* (*S.*) *minuta* ; (b) *S.* (*S.*) *africana* ; (c) *S.* (*S.*) *silvatica* ; (d) *S.* (*S.*) *squamipleuris*.

3. Subgenus *Spelaeomyia* **nov.** Type : *S. mirabilis* Parrot & Wanson.

Style with two spines of which one is terminal and with a small seta. Process with long hairs near base of coxite. Penis sheet rudimentary. Genital filaments short and thick with terminal process (fig. 15a). Legs very long. Buccal armature consisting of a row of long pointed teeth (fig. 15b). Pharynx unarmed. Spermathecae irregularly crinkled sack (fig. 15c). (Cave form.)

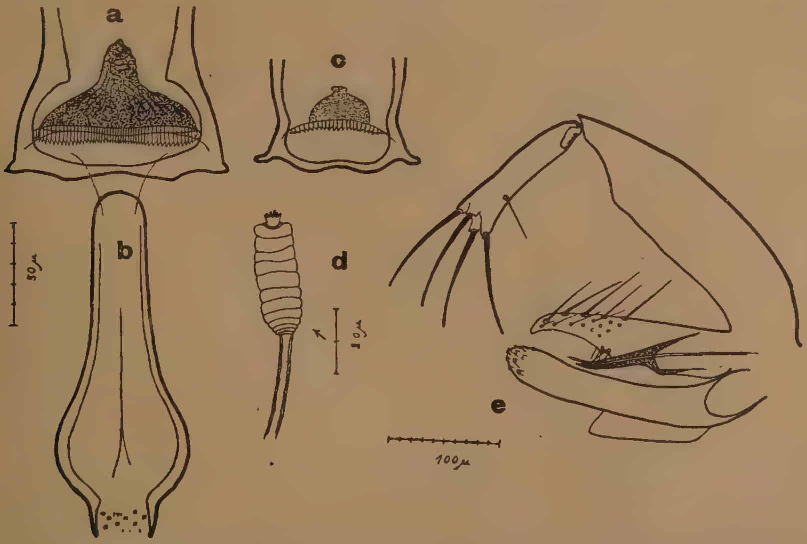

Fig. 14.—*Sergentomyia* (*Sintonius*) *wansoni* : (a) Buccal cavity of female ; (b) Pharynx of female ; (c) Buccal cavity of male ; (d) Spermatheca ; (e) Male genitalia.

Genus Lutzomyia França 1924.

Type : *Lutzomyia longipalpis* Lutz & Neiva 1912.

Buccal cavity with armature consisting of a row of long horizontal teeth and one or more rows of small vertical teeth. Bulge in dorsal wall of buccal cavity. Pharynx unarmed or armed. Male genitalia : style with three, four or five spines. Various appendages and hair tufts on parameres and coxite. Spermathecae of various forms. (America.)

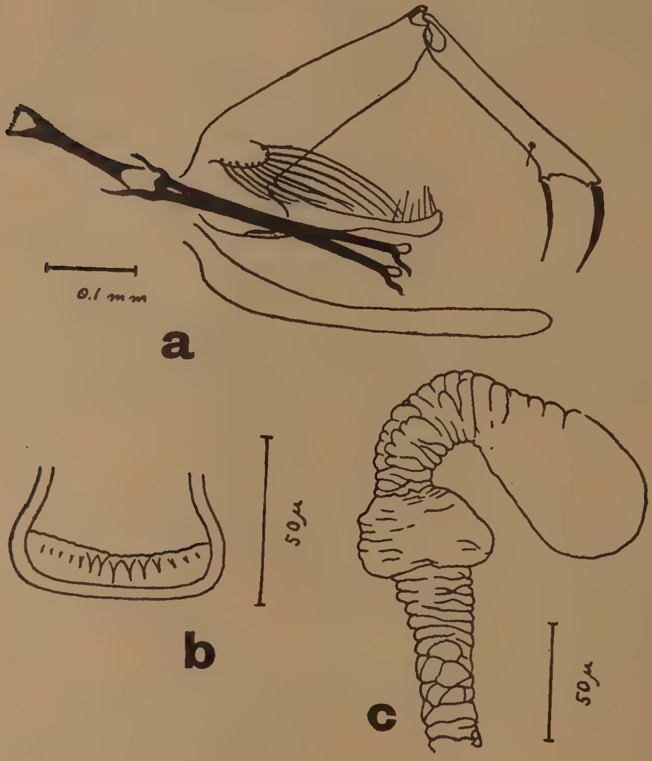

Fig. 15.—*Sergentomyia* (*Spelaeomyia*) *mirabilis* : (a) Male genitalia ; (b) Buccal cavity of female ; (c) Spermatheca (redrawn after Parrot).

NOTE.—All male genitalia are drawn to the same scale and in lateral view. Only one half shown.

Genus Brumptomyia França & Parrot 1921.

Type : *Brumptomyia brumpti* Larrousse 1920.

Armature in buccal cavity rudimentary. Style long and slender with five spines of which two are terminal and three nearer middle of segment. Tuft of hairs near base of coxite. Parameres simple. Spermathecae segmented or incompletely segmented. (America.)

KEY TO OLD WORLD SUBGENERA OF THE PHLEBOTOMINAE.

1. Armature in buccal cavity absent or rudimentary. Hairs on abdominal tergites mostly erect..(Genus *Phlebotomus*) 2

Armature in buccal cavity well developed. Hairs on abdominal tergites all recumbent or only a few erect...............................(Genus *Sergentomyia*) 9

2. Process with hairs near base of coxite..3

No such process..5

3. Process small with a few short hairs. Style long and cylindrical with three short spines terminal and two nearer middle of segment. Parameres with two long processes. Lateral lobes with short spatulate spines at tip. Pharynx armature appearing as network of lines. Spermathecae with equal segments and terminal knob.. Subgenus *Phlebotomus* Rond.

Process large with brush of long hairs. Apical part of paramere with flat elliptical upper surface..4

4. Style with four long spines of which two are near tip and two nearer base of segment. Spermathecae segmented with apical segment enlarged. Pharynx with armature of coarse teeth..................... Subgenus *Paraphlebotomus* **nov.**

Style with five long spines of which two are terminal and three nearer middle of segment. Apical segment of spermathecae not enlarged. Armature of pharynx appearing as irregular lines and small teeth................................ Subgenus *Synphlebotomus* **nov.**

5. Parameres two or three lobed. Pharynx with median armature of small teeth and posterior parallel ridges..6

Parameres simple..7

6. Style with five long spines, two terminal and three medial. Spermathecae segmented or striated with terminal segment defined and enlarged.............. Subgenus *Euphlebotomus* **nov.**

Style with four spines, two near tip, two nearer middle of segment. Apical segment of spermathecae not enlarged.......... Subgenus *Anaphlebotomus* **nov.**

7. Style with three long spines of which two are terminal and one near middle of segment. Penis sheet rudimentary, genital filaments very short and thick. Spermathecae incompletely segmented.... Subgenus *Australophlebotomus* **nov.**

Style with four long spines of which one is terminal and the three others at various intervals along the segment. Small seta on inner aspect also present. Eyes reduced, antennae, legs, and male genitalia much elongated. Pharynx with a few scale-like teeth. Spermathecae wide, ducts ending in narrow tubes. (Cave form)............................Subgenus *Spelaeophlebotomus* **nov.**

Style with five long spines, two terminal and three medial............... 8

8. Pharyngeal armature consisting of numerous small point-like teeth. Spermathecae segmented with long terminal process................................ Subgenus *Larroussius* Nitz.

Pharyngeal armature triangular or rounded, consisting of teeth of medium size. Spermathecae incompletely segmented................. Subgenus *Adlerius* Nitz.

9. Style with two spines of which one is terminal and small seta on inner aspect also present. Legs very long. Spermathecae irregularly crinkled sack. (Cave form)... Subgenus *Spelaeomyia* **nov.**

Style with four long spines of which at least two are terminal and small seta on inner aspect also present*...10

10. All abdominal hairs recumbent. Spermathecae either smooth or crinkled tubes or smooth capsules with or without spines....................................... Subgenus *Sergentomyia* França & Parrot

Some abdominal hairs erect, others recumbent. Spermathecae segmented or crenulated.. Subgenus *Sintonius* Nitz.

LIST OF SPECIES OF THE OLD WORLD.

GENUS PHLEBOTOMUS.

Subgenus Phlebotomus.

1. *P. papatasi*, Scopoli, 1786, Del. Faun. Flor. Insubr.
   Mediterranean, Sudan, West Asia.
   (*a*) var. *bergeroti*, Parrot, 1934, Arch. Inst. Pasteur Algérie, **12,** p. 383.
   P. viduus, Parrot, 1936, *ibid.*, **14,** p. 30.
   Sahara, Abyssinia, Sudan.
2. *P. roubaudi*, Newstead, 1913, Bull. Soc. Path. exot., **6,** p. 124.
   Sahara, Tropical Africa.
   Doubtful Species.
   P. duboscqi, Neveu-Lemaire, 1906, Bull. Soc. zool. France, **31,** p. 64.
   Tropical Africa, Sahara.
   (This species is possibly identical with either *P. papatasi* or *P. roubaudi*. See Parrot (1940) Arch. Inst. Pasteur Algérie, **22,** p. 40.)

Subgenus Paraphlebotomus nov.

3. *P. alexandri*, Sinton, 1928, Indian J. med. Res., **16,** p. 308 (*P. sergenti* var. *alexandri*).
   Newstead, 1920, Bull. ent. Res., **11,** p. 307 (*P. sergenti* var.).
   Parrot, 1936, Arch. Inst. Pasteur Algérie, **14,** p. 428 (*P. alexandri*, Sinton).
   West Asia, Sudan, North Africa.
4. *P. caucasicus*, Marzinowsky, 1917, Med. Rev. Moscow, **89,** p. 612.
   P. li, Popoff, 1926, Arch. Schiffs- u. Trop.-Hyg. Leipzig, **30,** p. 240.
   Turkestan, Persia.
5. *P. jacusieli*, Theodor, 1947, Bull. ent. Res., **38,** p. 95, ♂ only.
   Northern Palestine.
6. *P. maris-mortui*, Theodor, 1947, Bull. ent. Res., **38,** p. 92.
   Southern Palestine.
7. *P. mongolensis*, Sinton, 1928, Indian J. med. Res., **16,** p. 309 (*P. sergenti* var. *mongolensis*).
   P. sergenti var., Patton & Hindle, 1926, Proc. roy. Soc., (**B**) **100,** p. 408.
   China.
8. *P. sergenti*, Parrot, 1917, Bull. Soc. Path. exot., **10,** p. 564.
   Mediterranean, Sahara, West Asia.
   (*a*) var. *saevus*, Parrot & Martin, 1939, Arch. Inst. Pasteur Algérie, **17,** p. 484.
   Abyssinia, Sudan.

Subgenus Synphlebotomus nov.

9. *P. eleanorae*, Sinton, 1931, Indian J. med. Res., **18,** p. 817, ♂ only.
   India.
10. *P. katangensis*, Bequaert & Walravens, 1930, Rev. Zool. Bot. afr., **19,** p. 34, ♂ only.
    Belgian Congo.
11. *P. martini*, Parrot, 1936, Arch. Inst. Pasteur Algérie, **14,** p. 30.
    Abyssinia, Sudan.
12. *P. rossi*, De Meillon & Lavoipierre, 1944, J. ent. Soc. sthn Afr., **7,** p. 38, ♂ only.
    Southern Rhodesia.

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* A form with seven spines on the style has been included here as it is possibly an abnormality.

Subgenus Larroussius Nitzulescu, 1931.

13. *P. ariasi*, Tonnoir, 1921, Ann. Soc. ent. Belg., **61**, p. 53.
    West Mediterranean.

14. *P. kandelakii*, Shurenkova, 1939, Russ. J. trop. Med., **7**, p. 692.
    Lebanon, Persia, Turkestan.

15. *P. keshishiani*, Shurenkova, 1936, Med. Parasit., **5**, p. 892.
    Turkestan.

16. *P. langeroni*, Nitzulescu, 1930, Ann. Paras. hum. comp., **8**, pp. 382, 547.
    North Africa.
    (*a*) var. *orientalis*, Parrot, 1936, Arch. Inst. Pasteur Algérie, **14**, p. 30.
    Abyssinia, Sudan.

17. *P. longicuspis*, Nitzulescu, 1930, Ann. Paras. hum. comp., **8**, p. 547 (*P. langeroni* var. *longicuspis*, Nitz.).
    North Africa.

18. *P. longipes*, Parrot, 1939, Arch. Inst. Pasteur Algérie, **17**, p. 143.
    Abyssinia, Sudan.

19. *P. major*, Annandale, 1910, Rec. Indian Mus., **4**, p. 46.
    P. major var. *griseus*, Annandale, 1911, Rec. Indian Mus., **4**, p. 320.
    India, Mediterranean.
    (*a*) var. *syriacus*, Adler & Theodor, 1931, Proc. roy. Soc., (**B**) **108**, p. 447.
    Syria, Palestine.

    Various Mediterranean forms of *P. major* will eventually have to be separated, possibly as species, in which case the name *P. neglectus* Tonnoir, 1922, will have to be re-instated.

20. *P. perfiliewi*, Parrot, 1930, Arch. Inst. Pasteur Algérie, **8**, p. 383.
    P. macedonicus, Adl. & Theod., 1931, Proc. roy. Soc., (**B**) **108**, p. 447.
    Parrot, 1935, Arch. Inst. Pasteur Algérie, **13**, p. 257.
    Mediterranean.
    (*a*) var. *transcaucasicus*, Perfiliew, 1937, Faune de l'U.R.S.S., Dipt. III, no. 2, p. 108.
    Azerbaidjan.

21. *P. perniciosus*, Newstead, 1911, Bull. ent. Res., **2**, p. 47.
    P. legeri, Mansion, 1913, Bull. Soc. Path. exot., **6**, p. 639.
    P. lusitanicus, França, 1918, *Ibid.*, **11**, p. 732.
    ?*P. mascittii*, Grassi, 1908, Atti Accad. Lincei, **17**, p. 681.
    West Mediterranean.

    P. perniciosus is possibly identical with *P. mascittii*, Grassi, 1908. Parrot (1944), however, considers *P. larroussei* as identical with *P. mascittii*. This question can only be settled by a re-examination of Grassi's types.

22. *P. smirnovi*, Perfiliew, 1941, Trans. Acad. milit. med. Kiroff, **25**, p. 272 (description not available).
    Turkestan.

23. *P. tobbi*, Adler & Theodor, 1930, Bull. ent. Res., **21**, p. 559 (*P. perniciosus* var. *tobbi*).
    Parrot, 1934, Arch. Inst. Pasteur Algérie, **12**, p. 82 (*P. tobbi*, Adl. & Theod.).
    P. pirumowi, Burakova & Mirzayan, 1934, Trans. Counc. Study Indust. Resources, Ser. Transcaucas., pt. 11, p. 81.
    East Mediterranean, Persia.

24. *P. wenyoni*, Adler & Theodor, 1930, Bull. ent. Res., **21**, p. 559.
    Persia, Turkestan.

Subgenus Adlerius Nitzulescu.

25. *P. chinensis*, Newstead, 1916, Bull. ent. Res., **7**, p. 191 (*P. major* var. *chinensis*).
    China.
    (*a*) var. *longiductus*, Parrot, 1928, Arch. Inst. Pasteur Algérie, **6**, p. 29 (*P. major* var. *longiductus*, Parrot).
    Bokhara.
    (*b*) var. *simici*, Nitzulescu, 1931, Ann. Paras. hum. comp., **9**, p. 261.
    Jugoslavia, Palestine.

    There are at least four or five other forms of *P. chinensis* from India, Persia, Syria, Cyprus, and Rumania. All these forms will eventually have to be separated as varieties or species.

26. *P. larroussei*, Langeron & Nitzulescu, 1931, Ann. Paras. hum. comp., **9,** p. 72.
    P. vesuvianus, Adl. & Thd., 1931, Bull. ent. Res., **22,** p. 105.
    P. perniciosus var. *nitzulescui*, Simic, 1932, Ann. Paras. hum. comp., **10,** p. 431.
    Mediterranean.
    (*a*) var. *canaaniticus*, Adler & Theodor, 1931, Proc. roy. Soc., (**B**) **108,** p. 447 (*P. canaaniticus*, Adl. & Thd.).
    East Mediterranean.

Parrot (1944) considers *P. larroussei* as identical with *P. mascittii*, Grassi, according to Newstead's drawings. As pointed out above the identity of *P. mascittii* will have to be established by re-examination of Grassi's types.

Subgenus Euphlebotomus nov.

27. *P. argentipes*, Annandale and Brunetti, 1908, Rec. Indian Mus., **2,** p. 101.
    P. argentipes var. *marginatus*, Annandale, 1910, Spol. Zeyl., **7,** p. 57.
    India, Indochina.

28. *P. kiangsuensis*, Yao & Wu, 1938, Chin. med. J., Suppl. 2, p. 527.
    China.

29. *P. lesleyae*, Lewis & Kirk, 1946, Proc. R. ent. Soc. Lond., (**B**) **15,** p. 55.
    Sudan.

30. *P. newsteadi*, Sinton, 1926, Indian J. med. Res. **13,** p. 559.
    India.

The position of *P. newsteadi* in this group is provisional as the shape of the paramere as given by Sinton differs from that of the other species of this group. This may be an artefact due to mounting, however, and more specimens will have to be examined in order to establish the position of the species.

31. *P. philippinensis*, Manalang, 1930, Philipp. J. Sci., **41,** p. 175.
    Philippines.

Subgenus Anaphlebotomus nov.

32. *P. colabaensis*, Young & Chalam, 1927, Indian J. med. Res., **14,** p. 859.
    India.

33. *P. rodhaini*, Parrot, 1930, Rev. Zool. Bot. afr., **19,** p. 181, ♂ only.
    Belgian Congo, Sudan.

34. *P. stantoni*, Newstead, 1917, Bull. ent. Res., **5,** p. 190.
    P. maynei, Sinton, 1930, Indian J. med. Res., **18,** p. 195.
    India, Malaya, Indochina.

Subgenus Australophlebotomus nov.

35. *P. brevifilis*, Tonnoir, 1935, Bull. ent. Res., **26,** p. 137.
    Australia.

Subgenus Spelaeophlebotomus nov.

36. *P. gigas*, Parrot & Schwetz, 1937, Rev. Zool. Bot. afr., **29,** p. 221, ♀; 1938, *ibid.*, **31** p. 153, ♂.
    Belgian Congo, in cave.

GENUS SERGENTOMYIA FRANÇA, 1920.

Subgenus Sergentomyia, França, 1920.

1. *Group minuta.*

37. *S. minuta*, Rondani, 1843, Ann. Soc. ent. France, (1) **2,** p. 263.
    P. parroti var. *italicus*, Adler & Theodor, 1931, Bull. ent. Res., **22,** p. 105.
    Mediterranean.
    (*a*) var. *parroti*, Adler & Theodor, 1927, Ann. trop. Med. & Paras., **21,** p. 61 (*P. parroti*).
    North Africa, Cyprus, Crete.

38. *S. renauxi*, Parrot & Schwetz, 1937, Rev. Zool. Bot. afr., **29,** p. 222, ♀ only.
    Belgian Congo.

2. *Group fallax.*

Section (*a*).

✓ 39. *S. antennata*, Newstead, 1912, Bull. ent. Res., **3,** p. 365.
?*P. dubius*, Parrot, 1945, Arch. Inst. Pasteur Algérie, **23,** p. 232.
Tropical Africa.

The identity of this species is not clear and Newstead's type will have to be re-examined.

40. *S. azizi*, Adler, 1946, Bull. ent. Res., **36,** p. 497.
Cyprus.

41. *S. bruchoni*, Parrot, 1935, Arch. Inst. Pasteur Algérie, **13,** p. 249.
Greece.

42. *S. buxtoni*, Theodor, 1933, Bull. ent. Res., **24,** p. 537.
P. mathisi, Parrot, 1935, Arch. Inst. Pasteur Algérie, **13,** p. 239.
West Africa.

43. *S. cincta*, Parrot, 1944, Arch. Inst. Pasteur Algérie, **22,** p. 55, ♀ only. Perhaps a variety of *S. signatipennis.*
Abyssinia.

✓ 44. *S. congolensis*, Bequaert & Walravens, 1930, Rev. Zool. Bot. afr., **19,** p. 38 (*P. africanus* var. *congolensis*).
P. nairobiensis, Theodor, 1931, Bull. ent. Res., **22,** p. 469.
Tropical Africa.
✓ (*a*) var. *distincta*, Theodor, 1933, Bull. ent. Res., **24,** p. 237.
Tropical Africa.
(*b*) var. *firmata*, Parrot & Malbrant, 1945, Arch. Inst. Pasteur Algérie, **23,** p. 121.
Congo.

45. *S. cowlandi*, Lewis & Kirk, 1946, Proc. R. ent. Soc. London, (**B**) **15,** p. 55, ♀ only.
Sudan.
Probably identical with *S. congolensis.*

46. *S. dentata*, Sinton, 1933, Indian J. med. Res., **20,** p. 869.
P. minutus var. *arpaklensis*, Perfiliew, 1933, Zool. Anz., **101,** p. 221.
N.W. India, Turkestan.

47. *S. fallax*, Parrot, 1921, Bull. Soc. Hist. nat. Afr. N., **12,** p. 37 (*P. minutus* var. *fallax* Parrot).
N. Africa, Palestine.
3 503
(*a*) var. *cypriotica*, Adler, 1946, Bull. ent. Res., **~~2~~6,** p. ~~497~~.
Cyprus.

48. *S. occidentalis*, Theodor, 1933, Bull. ent. Res., **24,** p. 537 (*P. minutus* var *occidentalis*).
Perhaps identical with *S. antennata*, Newstead.
W. Africa.

49. *S. pastoriana*, Parrot, Mornet & Cadenat, 1945, Arch. Inst. Pasteur Algérie, **23,** p. 281. ♀ only.
W. Africa.

50. *S. punjabensis*, Sinton, 1933, Indian J. med. Res., **21,** p. 421 (*P. antennatus*, Newstead (Indian form)).
India.

51. *S. richardi*, Parrot & Wanson, 1946, Rev. Zool. Bot. afr., **39,** p. 225, ♀ only.
This is possibly a variety of *S. congolensis.*
Congo.

52. *S. schoutedeni*, Adler, Theodor & Parrot, 1929, Rev. Zool. Bot. afr., **18,** p. 70.
Tropical Africa.

✓ 53. *S. signatipennis*, Newstead, 1920, Bull. ent. Res., **11,** p. 305.
P. minutus var. *signatipennis*, Theodor, 1933, Bull. ent. Res., **24,** p. 537.
P. sanneri, Galliard & Nitzulescu, 1931, Ann.Paras. hum. comp., **9,** p. 233.
Mediterranean and Tropical Africa.

54. *S. simillimus*, Newstead, 1914, Bull. ent. Res., **5,** p. 179.
P. brodeni, Parrot, 1930, Rev. Zool. Bot. afr., **19,** p. 181.
Africa.

This species has been included here because of the structure of the pharynx which is typical for this group, although in other characters it shows affinities to other groups.

55. *S. theodori*, Parrot, 1942, Arch. Inst. Pasteur Algérie, **22,** p. 322.
    P. minutus, Adler & Theodor, 1926, Bull. ent. Res., **16,** p. 399.
    Palestine, Syria.

56. *S. yusafi*, Sinton, 1930, Indian J. med. Res., **18,** p. 171.
    East Africa.

Section (*b*).

57. *S. schwetzi*, Adler, Theodor & Parrot, 1929, Rev. Zool. Bot. afr., **18,** p. 72.
    P. symesi, Sinton, 1930, Indian J. med. Res., **18,** p. 171.
    P. schwetzi var. *aethiopicus*, Parrot, 1936, Arch. Inst. Pasteur Algérie, **14,** p. 30.
    Trop. Africa.

3. *Group africana.*

58. *S. africana*, Newstead, 1912, Bull. ent. Res., **3,** p. 363 (*P. minutus* var. *africanus*).
    Adler & Theodor, 1926, *ibid.*, **16,** p. 399.
    Africa.
    (*a*) var. *asiatica*, Theodor, 1933, Bull. ent. Res., **24,** p. 537.
    Palestine, N.W. India.
    (*b*) var. *atra*, Parrot, 1936, Arch. Inst. Pasteur Algérie, **14,** p. 30, ♀ only.
    Abyssinia.
    (*c*) var. *cherifiana*, Ristorcelli, 1939, Ann. Paras. hum. comp., **17,** p. 364.
    Morocco.
    (*d*) var. *eremitis*, Parrot & Jolinière, 1945, Arch. Inst. Pasteur Algérie, **23,** p. 56 (*P. eremitis*).
    Central Sahara.
    (*e*) var. *freetownensis*, ♀ only. Indian J. med. Res., **18,** p. 188 (*P. freetownensis*, Sinton).
    Sierra Leone.
    (*f*) var. *longior*, Parrot, 1936, Arch. Inst. Pasteur Algérie, **14,** p. 36.
    Abyssinia.
    (*g*) var. *magna*, Sinton, 1932, Indian J. med. Res., **20,** p. 565, ♀ only.
    Transvaal.
    This is possibly only a large specimen of *S. africana*.
    (*h*) var. *meridiana*, De Meillon & Lavoipierre, 1944, J. ent. Soc. sthn Afr., **7,** p. 38. ♀ only.
    Transvaal.
    (*i*) var. *nigra*, Parrot & Schwetz, 1937, Rev. Zool. Bot. afr., **29,** p. 221.
    Tropical Africa.
    (*j*) var. *sudanica*, Theodor, 1933, Bull. ent. Res., **24,** p. 537.
    Sudan, W. Africa.

59. *S. babu*, Annandale, 1910, Rec. Indian Mus., **4,** p. 49.
    India, Mauritius.
    (*a*) var. *insularis*, Theodor, 1938, Indian J. med. Res., **26,** p. 261.
    Ceylon.

60. *S. baghdadis*, Adler & Theodor, 1929, Ann. trop. Med. Paras., **23,** p. 269.
    Mesopotamia, N.W. India.

61. *S. barraudi*, Sinton, 1929, Indian J. med. Res., **16,** p. 716.
    Assam, Indochina.
    (*a*) var. *siamensis*, Causey, 1938, Amer. J. Hyg., **28,** p. 487, ♀ only.
    Siam.
    (*b*) var. *kwangsiensis*, Yao & Wu, 1941, Chin. med. J., **59,** p. 67.
    China.

62. *S. bigtii* Manalang, 1931, Philipp. J. Sci., **45,** p. 355.
    Philippines.

63. *S. crosarai*, Parrot & Wanson, 1946, Rev. Zool. Bot. afr., **39,** p. 225, ♀ only.
    Congo.

64. *S. dayapensis*, Manalang, 1931, Philipp. J. Sci., **45,** p. 355.
    Philippines.

65. *S. englishi*, Tonnoir, 1935, Bull. ent. Res., **26**, p. 137.
Australia.

66. *S. grekovi*, Khodukin, 1929, Meditz. Muisl' Uzbekisk. Turkmenist., suppl.
Turkestan.

67. *S. heiseri*, Manalang, 1930, Philipp. J. Sci., **42**, p. 299.
Philippines.

68. *S. himalayensis*, Annandale, 1910, Rec. Indian Mus., **4**, p. 35.
India.

69. *S. malabarica*, Annandale, 1910, Rec. Indian Mus., **4**, p. 48.
India.

70. *S. mangana*, Manalang, 1930, Philipp. J. Sci., **42**, p. 283.
Philippines.

71. *S. palestinensis*, Adler & Theodor, 1927, Ann. trop. Med. Parasit., **21**, p. 64, ♀; 1947, Bull. ent. Res., **38**, p. 96, ♂.
Palestine, Mesopotamia, Persia.

72. *S. queenslandi*, Hill, 1923, Bull. ent. Res., **14**, p. 83.
Australia.
(*a*) var. *meridionalis*, Tonnoir, 1935, Bull. ent. Res., **26**, p. 137.
Australia.

73. *S. shortti*, Adler & Theodor, 1927, Ann. trop. Med. Paras., **21**, p. 65.
India.

74. *S. sogdiana*, Parrot, 1928, Arch. Inst. Pasteur Algérie, **6**, p. 26; **7**, p. 303.
P. minutus var. *sogdianus*, Parrot, 1928, *Ibid.*
Turkestan.

75. *S. sumbarica*, Perfiliew, 1933, Zool. Anz., **101**, p. 221.
Turkestan.

76. *S. torrechantei*, Manalang, 1931, Philipp. J. Sci., **45**, p. 355.
Philippines.

77. *S. yvonnae*, Parrot & Schwetz, 1937, Rev. Zool. Bot. afr., **29**, p. 221, ♀ only.
Congo.

4. *Group zeylanica*

Section (*a*)

78. *S. arboris*, Sinton, 1931, Indian J. med. Res., **19**, p. 107, ♂.
Theodor *ibid.*, **26**, p. 261, ♂. India, Ceylon.

79. *S. khawi*, Raynal, 1936, Ann. Paras. hum. comp., **14**, p. 529.
China.

80. *S. koloshanensis*, Yao & Wu, 1946, J. Parasit., **32**, p. 87.
China.

81. *S. purii*, Sinton, 1931, Indian J. med. Res., **18**, p. 1203.
India.

82. *S. sylvestris*, Sinton, 1924, Indian J. med. Res., **11**, p. 1017.
P. demeijeri, Nitzulescu, 1930, Ann. Paras. hum. comp., **8**, p. 540.
India, Indo-China, Java.

83. *S. zeylanica*, Annandale, 1910, Spol. Zeyl., **7**, p. 60.
Theodor, 1938, Indian J. med. Res., **26**, p. 261.
P. chalami, Young & Chalam, 1927, Indian J. med. Res., **14**, p. 849.
India, Ceylon.

84. *S. ingrami*, Newstead, 1914, Bull. ent. Res., **5**, p. 179.
Adler, Theodor & Parrot, 1929 Rev. Zool. Bot. afr., **18**, p. 72.
S. rapta, Parrot, Mornet & Cadenat, 1945, Arch. Inst. Pasteur Algérie, **23**, p. 281.
Tropical Africa.

This species has been placed in this group, although the shape of the paramere is different from that of the other species of this group.

85. *S. serrata*, Parrot & Malbrant, 1945, Arch. Inst. Pasteur Algérie, **23**, p. 121, ♀ only.
Lewis & Kirk, 1946, Proc. R. ent. Soc. Lond., (**B**) **15**, p. 55, ♂.
Congo, Sudan.
Closely related to *S. ingrami* (Parrot, written communication).

86. *S. notata*, Parrot, 1938, Arch. Inst. Pasteur Algérie, **16**, p. 213, ♀ only.
Abyssinia.
The structure of the buccal cavity resembles that of *P. sylvestris*. Its position may have to be reconsidered when the male has been described.

Section (*b*)

87. *S. collarti*, Adler, Theodor & Parrot, 1929, Rev. Zool. Bot. afr., **18**, p. 72.
Belgian Congo.

88. *S. decipiens*, Theodor, 1931, Bull. ent. Res., **22**, p. 469.
Adler, Theodor & Parrot, 1929, Rev. Zool. Bot. afr., **18**, [p. 72 (*P. simillimus*, Newstead, 1914).
Belgian Congo, Sudan.

89. *S. dureni*, Parrot, 1934, Rev. Zool. Bot. afr., **24**, p. 266.
Congo.

90. *S. hitchensi*, Manalang, 1930, Philipp. J. Sci., **42**, p. 291.
Philippines.

91. *S. iyengari*, Sinton, 1933, Indian J. med. Res., **21**, p. 221.
India.
(*a*) var. *hainanensis*, Yao & Wu, 1938, 10th Congress Far East Ass. trop. Med. Hanoi, ♀ only.
Hainan Island.
(*b*) var. *hiberna*, Raynal & Gaschen, 1935, Bull. Soc. Path. exot., **28**, p. 582 (*P. hibernus*).
Theodor, 1938, Indian J. med. Res., **26**, p. 261.
Indo-China.
(*c*) var. *malayensis*, Theodor, 1938, Indian J. med. Res., **26**, p. 261.
Malaya.

92. *S. silvatica*, Raynal & Gaschen, 1935, Bull. Soc. Path. exot., **28**, p. 592.
Indochina.

93. *S. tonkinensis*, Raynal & Gaschen, 1935, Bull. Soc. Path. exot., **28**, p. 742, ♀ only.
Indochina.
The structure of the buccal cavity resembles that of *S. silvatica*. Whether *S. tonkinensis* belongs to section (*a*) or (*b*) of this group can only be decided after the male has been described.

5. *Group squamipleuris*

94. *S. pavlowsky*, Perfiliew, 1933, Zool. Anz., **101**, p. 221, ♀ only.
Turkestan.

95. *S. squamipleuris*, Newstead, 1912, Bull. ent. Res., **3**, p. 366.
P. ghesquieri, Parrot, 1929, Rev. Zool. Bot. afr., **18**, p. 90.
P. iraqi, Adler & Theodor, 1929, Ann. trop. Med. Paras., **23**, p. 269.
Tropical Africa, Mesopotamia.
(*a*) var. *dreyfussi*, Parrot, 1933, Arch. Inst. Pasteur Algérie, **11**, p. 603.
Sahara, Abyssinia.
(*b*) var. *indica*, Theodor, 1931, Bull. ent. Res., **22**, p. 469.
India, Turkestan.
(*c*) var. *inermis*, Theodor, 1938, Bull. ent. Res., **29**, p. 165.
Tropical Africa.

6. *Group nicnic*

96. *S. bailyi*, Sinton, 1931, Indian J. med. Res., **18**, p. 821.
India.
(*a*) var. *campestra*, Sinton, 1931, *ibid.*
India, Indo-China.

97. *S. hunti*, Lewis & Kirk, 1946, Proc. R. ent. Soc. Lond., (**B**) **15**, p. 55.
Sudan.

98. *S. kachekensis*, Yao & Wu, 1938, 10th Congress Far East Ass. Trop. Med. Hanoi, ♂ only.
China.

99. *S. nicnic*, Banks, 1919, Philipp. J. Sci., **14**, p. 163.
Philippines.

7. *Aberrant species*

100. *S. hirta*, Parrot & Jolinière, 1945, Arch. Inst. Pasteur Algérie, **23**, p. 56, ♂ only.
Central Sahara.

This species, which has been described from a single parasitised male, has 7 spines on the style. This is possibly an abnormality.

101. *S. horgani*, Lewis & Kirk, 1946, Proc. R. ent. Soc. Lond., (**B**) **15**, p. 55.
Sudan.

Closely related to *S. wurtzi*, with which it may be identical.

102. *S. montana*, Sinton, 1924, Indian J. med. Res., **11**, p. 807 ; **15**, pp. 21, 30.
India.

103. *S. ruttledgei*, Lewis & Kirk, 1946, Proc. R. ent. Soc. Lond., (**B**) **15**, p. 55, ♀ only.
Sudan.

104. *S. squamirostris*, Newstead, 1923, Ann. trop. Med. Paras., **17**, p. 531.
P. taianensis, Patton & Hindle, 1928, Proc. roy. Soc., (**B**) **102**, p. 533.
China.

Buccal and pharyngeal armature resemble that of *S. squamipleuris* but the spermathecae and male genitalia are quite different.

105. *S. wurtzi*, Parrot, 1938, Arch. Inst. Pasteur Algérie, **16**, p. 213.
Abyssinia.

8. *Insufficiently known or described species*

106. *S. morini*, Raynal & Gaschen, 1935, Bull. Soc. Path. exot., **28**, p. 731, ♂ only.
Indo-China.

107. *S. murgabiensis*, Perfiliew, 1939, Trav. Acad. Mil. Med. Kiroff, **19**, p. 75. (Description not available.)
Turkestan.

108. *S. perturbans*, de Meijere, 1909, Tijdschr. Ent., **52**, p. 191.
Java.

109. *S. pooi*, Yao & Wu, 1941, Chin. med. J., **59**, p. 67, ♂ only.
China.

110. *S. sequens*, Perfiliew, 1941, Trav. Acad. Mil. Med. Kiroff, **25**, p. 272. (Description not available.)
Crimea.

111. *S. viator*, Parrot, 1939, Arch. Inst. Pasteur Algérie, **17**, p. 143, ♂ only.
Abyssinia.

112. *S. bedfordi*, Newstead, 1914, Bull. ent. Res., **5**, p. 179.
S. Africa.

Parrot (written communication) recently re-examined a specimen from the locality from which *P. bedfordi* was originally described and which shows the short fifth palp segment considered characteristic for this species by Newstead. He considers it closely related to *P. congolensis*. For a definite identification of *P. bedfordi*, Newstead's type will have to be re-examined.

Subgenus Sintonius, Nitzulescu, 1931.

113. *S. adleri*, Theodor, 1933, Bull. ent. Res., **24**, p. 537.
W. Africa, Sudan.

114. *S. affinis*, Theodor, 1933, Bull. ent. Res., **24**, p. 537, ♀.
Lewis & Kirk, 1940, Proc. R. ent. Soc. Lond., (**B**) **9**, p. 127, ♂.
Sudan.

115. *S. caffrarica*, De Meillon & Lavoipierre, 1944, J. ent. Soc. sthn Afr., **7,** p. 38, ♀ only.
Cape Province.

116. *S. christophersi*, Sinton, 1927, Indian J. med. Res., **15,** pp. 21, 29, 33.
India.

117. *S. clydei*, Sinton, 1928, Indian J. med. Res., **16,** p. 179.
P. vagus, Parrot, 1939, Arch. Inst. Pasteur Algérie, **17,** p. 143.
N.W. India, Tropical Africa.

118. *S. eadithae*, Sinton, 1932, Indian J. med. Res., **20,** p. 577, ♀ only.
India.

119. *S. hodgsoni*, Sinton, 1933, Indian J. med. Res., **20,** p. 783.
N.W. India.

120. *S. hospitti*, Sinton, 1924, Indian J. med. Res., **12,** p. 261 (*P. simillimus* var. *hospitii*).
India.

121. *S. meilloni*, Sinton, 1932, Indian J. med. Res., **20,** p. 565.
South Africa.
(*a*) var. *suberecta*, Sinton, 1932, Indian J. med. Res., **20,** p. 565, ♂ only.
East Africa.

122. *S. subtilis*, Parrot, 1939, Arch. Inst. Pasteur Algérie, **17,** p. 143, ♂ ; **18,** p. 300 (1940), ♀.
Abyssinia.

123. *S. thomsoni*, Theodor, 1938, Bull. ent. Res., **29,** p. 165.
Nyasaland.

124. *S. tiberiadis*, Adler, Theodor & Lourie, 1930, Bull. ent. Res., **21,** p. 529.
Palestine.

125. *S. transvaalensis*, Sinton, 1933, Indian J. med. Res., **20,** p. 879, ♀ only.
Transvaal.

126. *S. wansoni*, Parrot, 1938, Rev. Zool. Bot. afr., **30,** p. 361, ♂ ; **32** (1940) p. 145, ♀.
P. matadiensis, Theodor, 1938, Bull. ent. Res., **29,** p. 165.
Congo, W. Africa.

Subgenus Spelaeomyia nov.

127. *S. mirabilis*, Parrot & Wanson, 1939, Rev. Zool. Bot. afr., **32,** p. 149.
Belgian Congo, in cave.

References.

The references given below are only those concerned with classification. References relating to the descriptions of species are given in the list of species.

ADLER, S. & THEODOR, O. (1926). On the *minutus* group of the genus *Phlebotomus* in Palestine.—Bull. ent. Res., **16,** p. 399.

—— & ——. (1927). On a collection of *Phlebotomus* sp. of the *minutus* group.—Ann. trop. Med. Parasit., **21,** p. 61.

—— & ——. (1929). The distribution of sandflies and leishmaniasis in Palestine, Syria and Mesopotamia.—Ann. trop. Med. Parasit., **23,** p. 269.

ALEXANDER, C. P. (1929). A revision of the American two-winged flies of the Psychodid sub-family Bruchomyinae.—Proc. U.S. nat. Mus., **75,** art. 7, p. 1.

CHRISTOPHERS, S. R. & BARRAUD, P. J. (1926). The development of the male and female hypopygium of *Phlebotomus*.—Indian J. med. Res., **13,** p. 853.

COSTA, G. (1843). (Quoted after Grassi.)—Ann. Accad. Aspir. Nat., **1,** p. 4.

COSTA LIMA, A. DA (1932). Sobre os phlebotomos americanos.—Mem. Inst. Osw. Cruz, **26,** p. 15.

DYAR, H. G. (1929). The present knowledge of the American species of *Phlebotomus* Rondani.—Amer. J. Hyg., **10,** p. 112.

Feuerborn, H. J. (1922). Der sexuelle Reizapparat der Psychodiden.—Arch. Naturgesch., **88,** Abt. A, p. 1.

França, C. (1919). Observations sur le genre *Phlebotomus* I.—Brotéria, Ser. Zool. **17,** p. 102.

——. (1920). Observations sur le genre *Phlebotomus* II. Phlébotomes du nouveau monde.—Bull. Soc. portug. Sci. nat., **8,** p. 215.

—— & Parrot, L. (1920). Introduction à l'étude systématique des diptères du genre *Phlebotomus*.—Bull. Soc. Path. exot., **13,** p. 695.

—— & ——. (1921). Essai de classification des Phlébotomes.—Arch. Inst. Pasteur Afr. N., **1,** p. 279.

Grassi, B. (1907). Ricerche sui flebotomi.—Mem. Soc. ital. Sci. nat., (3) **14,** p. 353.

Handlirsch, A. (1925). In Schröder (C.) Handbuch der Entomologie, **3,** p. 91.

Jobling, B. (1928). The structure of the head and mouth parts in *Culicoides pulicaris* L.—Bull. ent. Res., **18,** p. 211.

Kirk, R. & Lewis, D. J. (1946). Taxonomy of the Ethiopian Sandflies (*Phlebotomus*).
1. Classification and synonymy.—Ann. trop. Med. Parasit, **40,** p. 34.
2. Keys for the identification of the Ethiopian species.—*Ibid.*, p. 117.

Larrousse, F. (1921). Etude systématique et médicale des phlébotomes.—Thèse, Fac. Méd., Paris.

Magnitsky, W. J. & Gutzewitsch, A. W. (1929). Zur Frage über die Veränderlichkeit einiger systematischer Merkmale der Arten der Gattung *Phlebotomus*. —Zbl. Bakt., (1) Orig., **110,** p. 199.

Newstead, R. (1911). The papataci flies (*Phlebotomus*) of the Maltese Islands.—Bull. ent. Res., **2,** p. 47.

Nitzulescu, V. (1931). Essai de classification des phlébotomes.—Ann. Paras. hum. comp., **9,** p. 271.

Parrot, L. (1934). Sur les phlébotomes du groupe *minutus* et sur la classification des phlébotomes en général.—Arch. Inst. Pasteur Algérie, **12,** p. 389.

Rapp, W. F. (1944). Catalogue of North American Psychodidae.—J. N. Y. ent. Soc., **72,** p. 201.

Raynal, J. (1935). Contribution à l'étude des phlébotomes d'Indochine. Généralités.—Arch. Inst. Pasteur Indochine, no. 19, p. 337.

Rondani, C. (1843). Species italicae generis Hebotomi, etc.—Ann. Soc. ent. Fr., (2) **1,** p. 263.

Sinton, J. A. (1928). The synonymy of the Asiatic species of *Phlebotomus*.—Indian J. med. Res., **16,** p. 297.

Snodgrass, R. E. (1935). Principles of insect morphology. McGraw-Hill, New York.

Taylor, F. H. (1915). A blood-sucking species of *Pericoma* in Queensland (Dipt.). —Bull. ent. Res., **6,** p. 267.

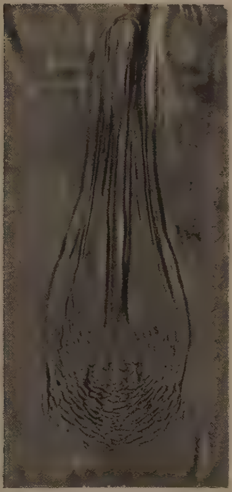
Theodor, O. (1932). Ueber eine neue Methode zur Klassifizierung von Phlebotomen und ihre Bedeutung für das Studium der durch Phlebotomen übertragenen Krankheiten.—Arch. zool., Torino, **16,** p. 1340.

——. (1932a). Sobre sistemática de los flebótomos sudamericanos.—7. Reun. Soc. argent. Pat. reg. Norte, Tucumán 1931, **2,** p. 764.

Tonnoir, A. L. (1933). Descriptions of remarkable Indian Psychodidae and their early stages, etc.—Rec. Indian Mus., **35,** p. 53.

——. (1935). The Australian species of the genus *Phlebotomus*.—Bull. ent. Res., **26,** p. 137.

Wanson, M. (1942). Sur la biologie des phlébotomes congolais.—Rec. Trav. Sci. méd. Congo belge, **1,** p. 23.



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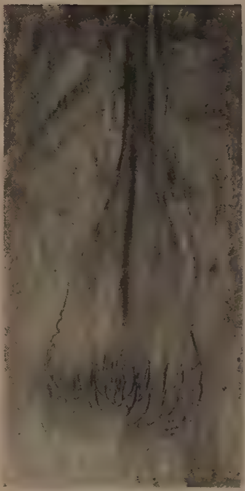

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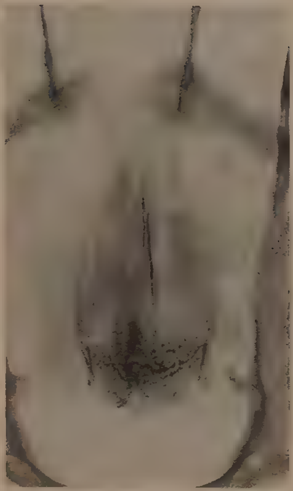

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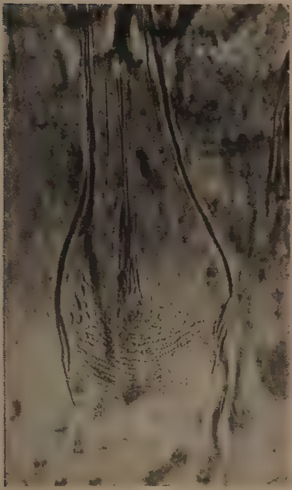

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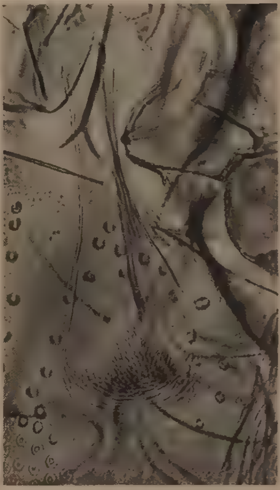

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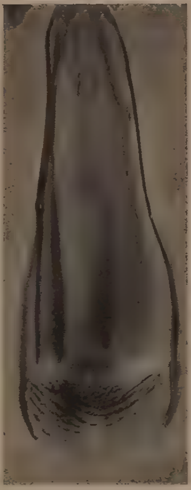
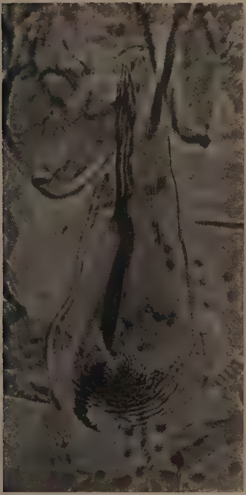


Fig. 6.

Fig. 1. Pharynx of female of *Phlebotomus* (*P.*) *papatasi*. (x 292)
Fig. 2. Same of *Phlebotomus* (*Paraphlebotomus*) *sergenti*. (x 292)
Fig. 3. Same of *Phlebotomus* (*Synphlebotomus*) *martini*. (x 358)
Fig. 4. Same of *Phlebotomus* (*Larroussius*) *major*. (x 292)
Fig. 5. Same of *Phlebotomus* (*Adlerius*) *chinensis* var. *simici*. (x 292)
Fig. 6. Same of *Phlebotomus* (*Euphlebotomus*) *argentipes*. (x 358)



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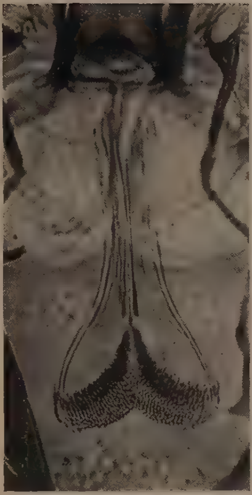

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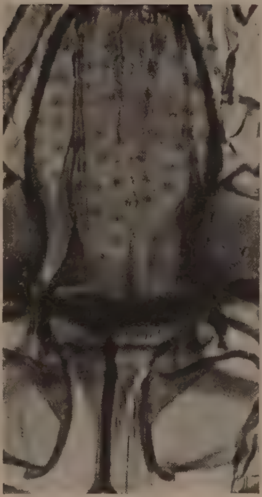

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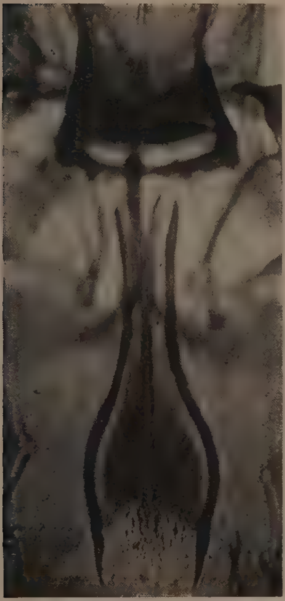

Fig. 4.

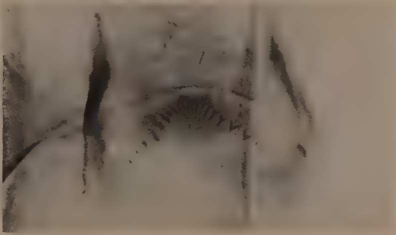

Fig. 5.

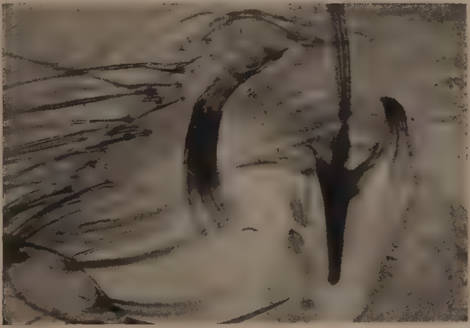

Fig. 6.

Fig. 1. Pharynx of female of *Phlebotomus (Anaphlebotomus) stantoni.* (x 358)
Fig. 2. Same of *Sergentomyia (S.) theodori.* (x 333)
Fig. 3. Buccal cavity of female of *Sergentomyia (S.) squamipleuris.* (x 333)
Fig. 4. Buccal cavity and pharynx of female of *Sergentomyia (S.) minuta.* (x 333)
Fig. 5. Buccal cavity of female of *Sergentomyia (S.) theodori.* (x 584)
Fig. 6. Process with hairs at base of coxite of male of *Phlebotomus (Paraphlebotomus) sergenti.* (x 333)

DEVELOPMENT OF *EPHESTIA ELUTELLA*, HB. (LEP., PHYCITIDAE) ON SOME NATURAL FOODS.

By N. Waloff, Ph.D. E.H.I

The primary aim of these experiments was to obtain data for the study of a natural population of *Ephestia elutella* breeding on bulk Manitoba wheat (Richards & Waloff, 1946). Observations of the life histories on other natural foods were made to detect any differences in the behaviour of the feeding larvae which might help in interpreting their behaviour on bulk wheat (Waloff & Richards, 1946).

Under warehouse conditions 98–99 per cent. of the larvae enter diapause after feeding, and remain at rest for 8–9 months. Unless otherwise stated, a selected stock, bred on middlings and not diapausing at 24–25°C. (E. B. Basden, unpublished) was used. This helped in isolating some of the diapause inducing factors.

The basic food requirements of this species have been analysed by Fraenkel and Blewett (1943, 1946a, 1946b), to whom I am grateful for useful suggestions.

Development of *E. elutella* Larvae on Manitoba Wheat.

Three sets of 75 freshly hatched larvae were placed one at a time in 3 in. × 1 in. tubes, each containing 150 whole Manitoba No. 1 wheat grains conditioned to 70 per cent. R. H. at 25°C. Each lot of grain was weighed at the beginning of the experiment, and 3 controls, for the possible alteration in grain weight, were included, making 78 tubes in each set. The tubes were kept in desiccators with 70 per cent. R.H. KOH solution, at 25°C., 21°C. and 17°C. At the end of the life histories, the emerged moths were weighed and sexed, the webbing wiped off the grain, frass sieved off, the grain reconditioned to 70 per cent. R.H. and reweighed. The loss in weight, the number of grains attacked, and the nature of the damage was noted.

The effect of temperature on life-history.

(i) The results of this set of experiments are summarised in Table I. The effect of the lowering of temperature, when relative humidity remained constant, was to prolong the feeding stage of the larvae and to induce diapause at 21°C. and 17°C. Whereas the total larval life at 25°C. lasts 35·9 days on the average, the corresponding feeding period preceding wandering and pupation or diapause at 21°C. and 17°C. lasts 65 and 66·9 days respectively. This closely coincides with the warehouse observations, where the egg incubation and feeding larval stages last 60–70 days at the average grain temperature of 16·9°C. The pupal stage was similarly prolonged by the fall in temperature. At 25°C., 70 per cent. R.H. it lasted 14 days on the average, whereas at 21°C. and 17°C. it was prolonged to 21 days. It may last 30–40 days in warehouse conditions, at the time preceding moth emergence in May or June. It is variable even at constant temperatures, for example at 25°C. it lasts 12–14 days in the majority of larvae, but occasionally it is as short as 6 days, and in one pupa it was as long as 37 days. The effect of temperature on the longevity and fecundity of the adults will be discussed in another paper (Waloff, Norris and Broadbent, in preparation).

No larvae diapaused on Manitoba wheat at 25°C., 70 per cent. R.H., but diapause was induced by the constant lower temperatures of 21°C. and 17°C., *i.e.*, the larvae spun up and remained quiescent for periods up to 400 days. This result was somewhat anomalous, as 59 out of 64 larvae (92·1 per cent.) entered diapause at 21°C., and only 19 out of 44 (43·2 per cent.) at 17°C.

TABLE I.

Development of *E. elutella* larvae on Manitoba No. 1 wheat.

Temperature (Humidity 70 per cent R.H.)	Egg incubation in days	Survival	Per cent. in diapause	Average length of larval life in days				Average length of pupal stage in days	Average No. days from egg hatching to emergence of moth		Average No. of grains attacked			Average weight in mg. eaten by a single larva			Average weight of a moth in mg.	
				Before spinning up	Spun up	Total days			Non-diapausing	Diapausing	No. full embryos eaten	Embryos punctured	No. grains attacked	♀	♂	Av. of ♀ and ♂	♀	♂
						Non-diapausing larvae	Diapausing larvae											
25°C.	4–5	65/75 86·6%	0	—	—	35·9 [limits 29–50]	—	13·8 [limits 6–37]	49·8 [limits 42–79]	—	47	5	52	73·3 (from 30 ♀)	74·4 (from 32 ♂)	73·3 (from 65 larvae)	9·7 (from 30 ♀)	7·7 (from 32 ♂)
21°C.	9–10	64/75 85·3%	92·1	65	196·5	64 [limits 50–80]	249·4 [limits 100–375]	21·3 limits [7–46]	84 [limits 71–102]	282·3 [limits 123–402]	49	4	53	81·3 (from 31 ♀)	87·7 (from 33 ♂)	84·6 (from 68 larvae)	10·6 (from 31 ♀)	8·9 (from 33 ♂)
17°C.	13–15	44/75 60%	43·2	66·9	192·6	61·2 [limits 54–80]	272·2 [153– 379]	20·6 [limits 6–39]	79·6 [limits 62–118]	294·7 [limits 151–401]	46	3	49	93·7 (from 22 ♀)	90·4 (from 22 ♂)	92·8 (from 49 larvae)	10·1 (from 22 ♀)	8·1 (from 22 ♂)

(ii) The amount of food eaten by larvae increased with the fall in temperatures, whereas the weights of the moths did not increase correspondingly. The average weights of moths at 21° and 17°C. were greater than at 25°C., but the weights at 21°C. were also slightly greater than at 17°C. At 25°C. the amount of food eaten by a female larva was 7·56 times, at 21°C. 7·67 times and at 17°C. 9·28 times the weight of the ♀ moth. The corresponding figures at 25°C., 21°C. and 17°C. for the males are 9·66, 9·85 and 11·16. This suggests that the utilisation of food is similar at 25°C. and 21°C., but seems less efficient at 17°C. The amounts eaten by the male larvae were equal to or greater than those consumed by the females. The weights of the female moths, on the other hand, were consistently greater than those of the males. A similar result is quoted by Goldschmidt from Farkas (1923), namely that in the silkworm the body weight of the female is not only absolutely higher than that of the male, but also relatively, that is, compared with the original weight of the caterpillars. He also states that the male caterpillars use more substance and energy than the female during a definite period, and for every gramme of substance used the male consumes 7·024 calories and the female 6·620.

In *Ephestia elutella*, the average weight of the fully fed male larvae in diapausing warehouse stock is equal to or greater than that of the female, yet the weight of the male moths is always lower (see Table II). It thus appears that more larval substance is needed to produce a male than a female adult.

TABLE II.

Stock	No.	Sex	Average weight of fully fed larvae in mg.	Average weight of moths in mg.	Moth weight as a percentage of larval weight
Diapausing warehouse stock kept in an unheated outhouse	85 62	♂ ♀	17·3 16·9	6·35 7·37	36·6 43·6
Diapausing warehouse stock kept at 25°C. 70 per cent. R.H.	43 51	♂ ♀	15·72 14·7	6·29 6·84	40·0 46·6
Non - diapausing stock, kept at 25°C., 70 per cent. R.H.	13 11	♂ ♀	11·6 14·6	6·3 9·5	54·3 65·4

(iii) Only the embryo is obviously attacked in Manitoba wheat. The 1st instar larvae nibble the pericarp and eat their way into the grain, leaving a neat puncture. They leave the embryos before moulting, but will often return to the same grain afterwards. Usually the 3rd instar larvae are too large to fit inside the embryos, and the rest of the feeding stage is passed outside the grains. Little, if any, of the endosperm appears to be eaten, unless there is a shortage of food. The embryo is neatly scooped out, and generally there are no rough edges to the pericarp. *E. elutella* damage can also be recognised by webbing with frass adhering to it. Fresh frass is white, but becomes brown on drying. The number of embryos eaten appears to be independent of temperature, and for 183 larvae at the temperatures of 17°C., 21°C. and 25°C. averaged 47·71±1·04 (standard error). As the amount of food by weight increases at lower temperatures, it must be that the embryos are more thoroughly scooped out and some of the endosperm is attacked. The possibility of the choice of larger grains cannot be dismissed altogether. I am grateful to Dr. O. W. Richards and Mr. T. A. Oxley, for the permission to quote their unpublished data on the choice of grains of different sizes by *E. elutella* larvae. A sample of grain, brought back from the granary, was sieved through a series of different meshes, the grain was weighed, and the percentage damage of each class was estimated. The results of this experiment are summarised in Table III. There appears to be little doubt that the larvae tend to attack the smaller grains, although these represent only a low percentage of the grain bulk.

TABLE III.

Grain size		Weight of grain in g.	Same as percentage of total weight	Number of damaged grains in 500	Percentage of damage in each sample
Passing sieve 7 meshes to inch	Held by sieve 7 meshes to inch				
—	6	32	2·67	23	4·6
6	7	336	28·10	32	6·4
7	8·5	656	54·90	54	10·8
8·5	<10	150	12·55	90	18·0
<10	>10	21*	1·76	56	11·2
Total ...		1,195			

* At least 50 per cent. broken grains.

Development of *E. elutella* Larvae on other Natural Foods.

E. elutella has been reported as a pest from a wide range of stored foods as different as tobacco, dry fruit, cacao, groundnuts, soya beans, wheat, etc. The rate of development on some of these foods has been traced and is summarised in Table IV. As a non-diapausing stock was used, it was of interest to note not only the foods on which the percentage of survival was high and development rapid, but also those which induced diapause. The natural foods fall into 3 groups :—

(*a*) Foods on which there was no diapause at 25°C., 70 per cent. R.H.

(*b*) Foods on which a low percentage of surviving larvae entered diapause at 25°C., 70 per cent. R.H.

(*c*) Foods on which most, or all of the surviving larvae entered diapause at 25°C., 70 per cent. R.H.

(*a*) *Foods on which there was no diapause at 25°C., 70 per cent. R.H.* included wheat germ, Manitoba wheat, unpolished rice, English oats, tobacco, figs and soya flour with 1·3 per cent. fat content. In Manitoba wheat and unpolished rice, the larvae fed on the embryos alone. The percentage survival on the first three foods was high and development rapid, there were fewer survivors on the other foods in this group. The most rapid development was on wheat germ, where the fully fed larvae wandered after 18 days, and the whole life history from hatching of the eggs to moth emergence averaged 41·6 days. Fraenkel and Blewett (1943, 1946a, 1946b) suggest that the rapid development on this food is due to the high vitamin B content (see Table V) and to the presence of linoleic acid, which is essential for the development of this species. Moreover, they state that pure wheat germ contains no starch, while its sugar content is at least 10 per cent. The implications of this are discussed later in paragraph (*c*).

TABLE IV.

Development of *E. elutella* on natural foods at 25°C., 70 per cent. R.H.

Food	Number of 1st instar larvae	Percentage survival (to moth emergence or diapause)	Total length of life (egg hatching to moth emergence in days)		Percentage of survivors diapause	Average weight of fully fed larvae mg.	Number weighed
			Non-diapausing	Dia-pausing			
GROUP (*a*) Manitoba No. 1 wheat*	75	86·6	49·8 (42–79)	—	0	13·0	14
Wheat embryo	20	100·0	41·6 (32–44)	—	0	—	—
Unpolished rice*	21	85·7	57·2 (47–76)	—	0	14·3	18
English oats with husks husks removed	375 25	14·4 52·0	80·4 (66–92)	—	0	12·6	18
Tobacco. Nyasa Brights. Flue cured	66	9·9	120 (86–175)	—	0	8·6	3
Figs	20	10·0	133·5 (121–146)	—	0	23·5	2
Soya flour. 1·3 per cent. fat	80	40·0	61 (53–72)	—	0	21·4	28
GROUP (*b*) Soya flour 7·3 per cent. fat	80	31·2	67·2 (59–72)	Over 288	32·0	17·2	20
Peas (dry) ...	34	20·6	82·1 (69–97)	258	14·3	18·0	1
Groundnuts cut up with shells	20	30·0	69 (58–97)	Over 255	33·3	17·8	11
Cacao beans whole... ... cut up ...	156 186	8·9 32·3	86·6 (63–122)	330 (175-449)	14·9	11·0	44
GROUP (*c*) Beans (dry) ...	32	6·2	0	376·5	100·0	18·6	3
White flour (N.S.R.)	20	25·0	137	Over 267	80·0	13·1	5
English wheat ...	30	46·6	0	259 to over 740	100·0	22·3	23
10 grains of Manitoba wheat	21	23·8	0	Over 284	100·0	—	—
Artificial diet with starch as carbohydrate	20	40·0	114·3	358 to over 428	75·0	—	—

* Only the embryos are eaten.

TABLE V.

Vitamin B content of some natural foods mg./100 g.

Food	Thiamin	Riboflavin	Nicotinic acid	Source
Venezuela cacao beans (whole)	·296			Cereals Research Station Min. of Food
Ceylon ,, ,, ,,	·25			
Cocoa	·09	·45	1·5	*N.R.C.
Groundnuts	·4	·13	16·0	N.R.C.
Patent flour	·07	·03	·6	†Williams
Wheat germ	2·1			‡Med. Res. Council
Beans	·45			,, ,,
Peas (dry)	·45			,, ,,
Soya-bean flour—(*a*) full fat	·66			,, ,,
,, ,, (*b*) low fat	·87			,, ,,

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* Tables of food composition. National Research Council, 1943. Washington.

† The University of Texas Publication No. 4237, 1942.

‡ Medical Research Council. Nutritive values of wartime foods. War Memorandum No. 14, 1945.

TABLE VI.

Composition and nutritive value of feeding stuffs.
Average composition per cent. as shown by analysis.

Food	Per cent. Water	Dry matter	Protein	Fat	Carbohydrate soluble	Carbohydrate fibre	Ash	Source
Wheat (embryo only, eaten)	—	86·6	12·1	1·9	69·0	1·9	1·7	*H. E. Woodman
Finest grade middlings ...	—	86·7	17·0	4·2	60·8	2·3	2·4	,,
Unpolished rice (embryo only, eaten)	—	88·6	8·3	1·8	64·7	8·8	5·0	,,
Oats	—	86·7	10·3	4·8	58·2	10·3	3·1	,,
Figs (dry)	—	70·8	3·3	0·4	58·4	6·4	2·3	,,
Peas	—	86·0	22·5	1·6	53·7	5·4	2·8	,,
Beans...	—	85·7	25·4	1·5	48·5	7·1	3·2	,,
Groundnuts	—	94·0	26·8	44·9	17·5	2·6	2·2	,,
Cacao beans (freshly roasted)	—	—	11·9	54·0 52·2–55·4	25·4		—	†A. W. Knapp ‡Bywaters
		(*Composition in g./100g.*)						
Wheat germ	8	—	32·0	7·7	37·8		—	§Med. Res. Council
Soya-bean flour 1–2 per cent. fat	—	—	42·2	1·0	15	available carbohydrate	—	,,
Soya-bean flour 7 per cent. fat	13	—	40·1	5·1	14		—	,,
White flour (70 per cent. English)	—	—	8·1	1·0	76·2		—	,,
White flour (70 per cent. Manitoba)	13	—	13·1	1·2	71·6		—	,,

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* Woodman, H. E. (1942). The composition and Nutritive Value of Feeding Stuffs. Bull. Min. of Agric. & Fisheries No. 124. H.M. Stationery Office.

† Knapp, A. W. Cocoa and chocolate. London, 1920.

‡ Bywaters, H. W. Modern methods of cocoa and chocolate manufacture. London, 1930,

§ Medical Research Council. Nutritive values of wartime foods. War Memorandum, No. 14. 1945.

Development on unpolished rice is also rapid, the larvae feeding on about 96 embryos. The low survival on English oats is at least partly caused mechanically, as many more larvae survive on grains with husks removed (Table IV). The embryo and the endosperm are completely eaten out, leaving the husks full of white frass. About 6 whole grains are required by a larva to complete its development. Both in unpolished rice and in English oats, the larvae wander freely throughout the food and their distribution is similar to that in bulk Manitoba wheat, where the majority of larvae remain in the upper 30 cm., but some penetrate to 150 cm. and lower.

The percentage survival on tobacco and on figs was very low, and although *E. elutella* is known to be a pest of dry fruit, attempts to rear it on raisins, dates and prunes in the laboratory have been unsuccessful. Both on figs and on tobacco the larvae did not diapause, but grew very slowly. The weights of the two survivors on figs were high—21 mg. and 26 mg. No larvae entered diapause on soya flour with 1·3 per cent. fat, whereas 32·0 per cent. diapaused on soya flour with 7·3 per cent. fat content.

(*b*) *Foods on which a low percentage of surviving larvae diapause at 25°C., 70 per cent. R.H.*—These included soya flour with 7·3 per cent. fat, cacao beans, groundnuts and dry peas. With the exception of dry peas, the fat content of these foods is high (Table VI). Of the larvae reared on cacao, only 8·9 per cent. survived on whole beans, and 32·3 per cent. on the cut up ones. As on English oats, the cause of low survival may be a mechanical one.

Five types of cacao beans were used in this experiment : Ceylon, Trinidad, West Africa, Bahia and Venezuela In the first four types, the larvae penetrated inside the beans, and fed on the " nib " (internal portion), while in Venezuela beans they remained outside, feeding on the outer coat and nibbling the cotyledons. Attempts to rear larvae on Ceylon cacao bean seed coats were unsuccessful—they all died—whereas 4 out of 10 larvae developed on Venezuela seed coats. Vitamin B_1 contents of whole Venezuela and Ceylon beans, of their seed coats and the " nib " have been kindly determined by the Cereals Research Station, Ministry of Food (Table VII). The optimum requirements of vitamins of B complex have been discussed by Fraenkel and Blewett (1946a) and it would seem that sufficient quantities of vitamin B_1 are present in all the cacao parts analysed. However, Vitamin B_1 of Venezuela cacao seed coats was found to be very high, and it is possible that this, among other factors, explains the more unusual feeding habits of the larvae on this type of cacao. The results of these observations are given in Table VII.

TABLE VII.

Food	Vit. B_1 mg./100 g. det. Cereals Research Station. Min. of Food	No. of larvae	No. Survived	Rate of larval development in days
Venezuela Cacao Bean Seed coat	1·2	10	4	2 larvae–115 days 2 diapaused over 343
Ceylon Cacao Bean Seed coat...	·5	10	0	0
" Nib " of Venezuela Cacao bean	·21	10	4	All diapaused over 343
" Nib " of Ceylon Cacao bean ...	·22	10	3	All diapaused over 343
Whole Venezuela Cacao bean (cut up)	·296	36	13	75·4, 0 per cent. diapause
Whole Ceylon Cacao bean (cut up)	·25	36	9	93·7, +22·1 per cent. in diapause

(*c*) *Foods on which a high percentage, or all of the surviving larvae entered diapause at 25°C., 70 per cent. R.H.*, were all characterised by a high starch content, and included English wheat, white flour (N.S.R.), dry beans, insufficient number of Manitoba wheat grains (10 instead of the required 48), and an artificial diet in which the only carbohydrate was starch. I am grateful to Dr. G. Fraenkel and Miss M. Blewett for supplying me with this basic diet.

On English wheat, which is softer than Manitoba, the larvae attack the starchy endosperm, as well as the embryo. On the average a larva requires about 12 grains of English wheat. All the fully fed larvae were heavy, and all the survivors entered diapause—two of them are still spun up after 740 days. The larvae with insufficient number of Manitoba wheat grains also attacked the endosperm, and again all the survivors entered diapause. On white flour (N.S.R.) the larvae grew very slowly, and 80 per cent. of them diapaused. Eight out of the 20 larvae reared on an artificial diet with starch as the only carbohydrate survived, but grew very slowly. Six entered diapause, one of these died after 330 days, the others diapaused for 358–428 days, whilst one is still spun up at the time of writing. Fraenkel and Blewett (1946a) discuss the need of about 50 per cent. of glucose in the diet of this species and its inability to utilise starch as a carbohydrate ; they also suggest that the limiting factor for *E. elutella*, even on wholemeal flour, is a lack of some vitamins of the B group.

Three sets of results became apparent in rearing the larvae on diets with a high starch content : (i) the percentage of survival was fairly low ; (ii) development was slow ; (iii) most, or all of the surviving larvae entered dispause.

The effect of a high percentage of starch on inducing diapause may, of course, be only a secondary one. It is more probable that this state is induced by the lack of some substance essential for the normal development of this stock of larvae. The work of Fraenkel and Blewett suggests that this substance is either glucose, or linoleic acid, or vitamins of B complex, or all these factors together.

It is interesting to note that moulting in the 4th or 5th instar of *Rhodnius prolixus* was delayed up to 111 days, instead of the usual 15 (Brecher & Wigglesworth, 1944) when the bug was reared in sterile conditions free from the symbiotic *Actinomyces rhodnii*. The authors suggest that growth ceases through some vitamin deficiency and that the vitamin is needed for the production of the moulting hormone. The same is possibly true of the production of pupation hormone in the larvae of *E. elutella*.

The Number of Instars of *E. elutella* Larvae.

(*a*) *The number of instars of* E. elutella *on Manitoba wheat at 25°C., 70 per cent. R.H.* The duration of larval instars in days, weights, and width of head capsules of successive instars have been determined. No difference in the rate of development and instar number has been noted in the laboratory and in the warehouse stocks (*i.e.*, in diapausing and non-diapausing larvae), but in every set of observations some of the larvae completed their development in 5 and some in 6 instars, under identical conditions, *i.e.*, when reared on Manitoba wheat at 25°C., 70 per cent. R.H. (One larva completed its development in 4 instars, and 7th instar larvae have also been recorded.) It is not known whether it is justifiable to refer to these differences as to " larval strains ", as no genetical experiments were carried out.

This difference in the number of larval instars made it impossible to take random samples for the measurements of head capsules and weights, and the life histories of 33 larvae were, therefore, traced individually. Eighteen of these larvae completed development in 5 instars and 15 in 6. The widths of head capsules, weights of some larvae immediately after moults and the rates of development are summarised in Tables VIII, IX and X and in figs. 1 and 2.

The 6 instar larvae take longer to develop than the 5 instar ones and while it is impossible to distinguish one set of fully grown larvae from one another, the ratio of the increase of the head capsule is smaller in the 6 instar larvae than in the 5. With practice it is possible to assign larvae to their instars by eye alone, but the most distinctive stage is the 5th instar of the 6 instar larvae, and it can be picked out with comparative ease. Roughly speaking, the growth curve follows

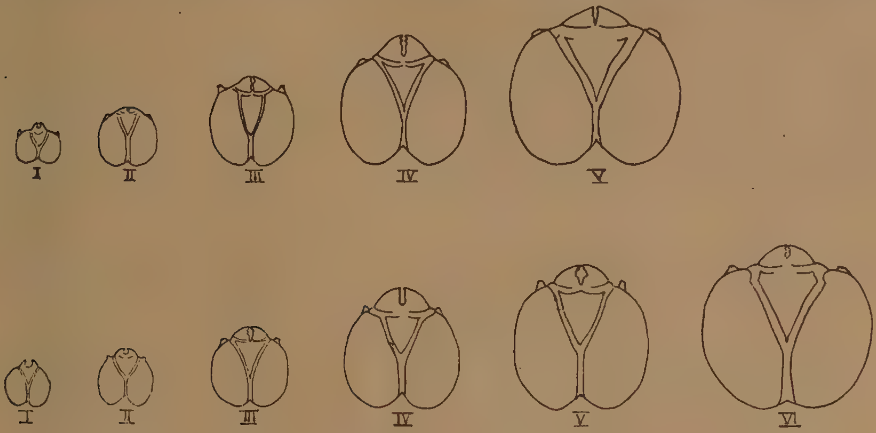


Fig. 1.—Progressive increase of head capsule width at each moult of V and VI instar larvae.

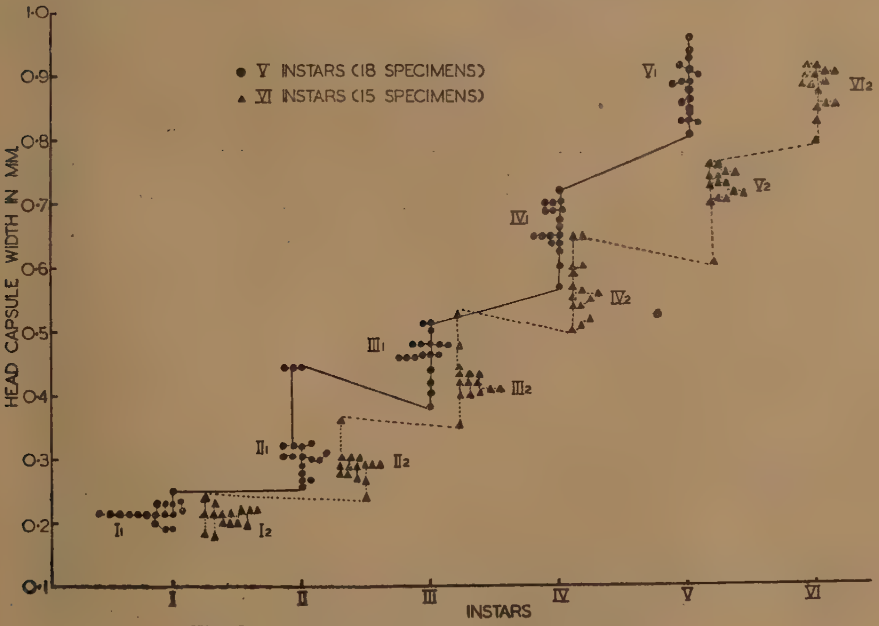


Fig. 2.—Head capsule widths of V and VI instar larvae.

Dyar's law (fig. 3), although it is more regular in 5 instar than in the 6 instar sets. The ratio of increase in head capsules does not remain a constant in either set of larvae, but decreases continuously in the 5 instar larvae and in the last 3 moults in the 6 instar ones. The difference between the number of larval instars is not linked with sex, as it is in *E. cautella*, where the males complete development after three moults and the females after four (Richards & Thomson, 1932).

TABLE VIII.

Width of head-capsules in mm.

No. of larvae	Instar					
	I	II	III	IV	V	VI
18 – V instar larvae ...	0·218 S.E. ±·0016	·325 ±·0067	·467 ±·0039	·665 ±·0043	·883 ±·0049	—
15 – VI instar larvae ...	0·212 S.E. ±·0022	·292 ±·0128	·430 ±·0054	·570 ±·0062	·729 ±·0049	·886 ±·0046

Increase expressed as ratio between successive instar head-capsules.

	I–II	II–III	III–IV	IV–V	V–VI
18 – V instar larvae	1·49	1·44	1·42	1·33	—
15 – VI instar larvae...	1·38	1·47	1·32	1·28	1·21

TABLE IX.

Average number of days	Number of moults and length of larval life in days					
	To 1st moult	To 2nd moult	To 3rd moult	To 4th moult	To 5th moult	Total larval life
20 – V instar larvae ...	7·5	12·7	20	26·9	—	36·4
Limits	6–11	11–18	16–25	21–35	—	—
12 – VI instar larvae ...	7·4	12·3	19·4	23·5	33·7	42
Limits	6–11	11–16	18–23	20–28	28–42	—

Most of the larvae collected in Manitoba wheat in the warehouse could be allocated to 6 instars.

Very occasionally larvae, apparently already in 6th instar, may moult during diapause. Two out of 253 in larvae kept in an unheated outhouse in Slough did so and three out of 225 of those kept at 25°C. These larvae were in no way disturbed. Cast head-capsules have also occasionally been seen near the hibernation sites of the larvae.

TABLE X.

Average wts. of 12 V instar larvae immediately after a moult.

	II After 1st moult	III After 2nd moult	IV After 3rd moult	V After 4th moult	At the end of feeding period
	·237 mg.	·762 mg.	3·905 mg.	9·331 mg.	18·625 mg.
Limits ...	·1–·375	·375–1·8	2·1–6·45	6·6–12·6	14–21

Average wts. of larval instars taken from cultures at regular intervals.

Instar	II	III	IV	V	VI
Average wt.	·322	·595	3·369	7·506	11·708
Limits ...	·21–·43	·10–1·2	1·4–6·2	2·8–16	7·0–16
No. larvae ...	5	11	16	17	12

(*b*) *The number of instars on Manitoba wheat at 25°C., 35·5 per cent. R.H.* At this low humidity the number of larval instars was considerably increased. The 20 larvae bred at this low humidity grew very slowly, and none of them survived. Most of the larvae died while they were still half grown, but one larva died after six moults, *i.e.* in instar seven, two died after seven moults, *i.e.* in instar eight, and one larva reached 12th instar after eleven moults. All the larvae died while still in the feeding stage, and the head capsules showed progressive increase in width after moults. The greatest widths do not exceed those of 5th and 6th instar larvae developing at the same temperature at 70 per cent. R.H. The number of moults in *Tineola* can be increased greatly on dry foodstuffs, but there moulting can take place without growth, and is even accompanied by decrease in size (Titschack, 1926).

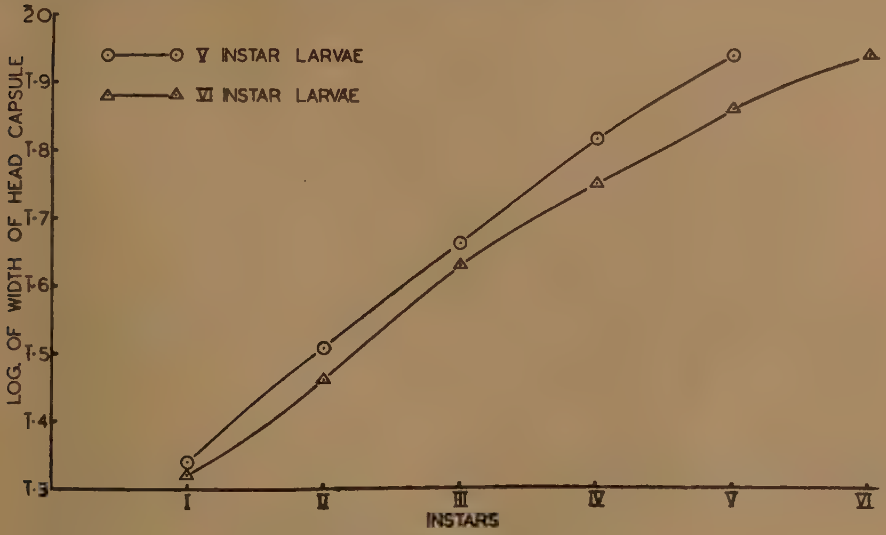
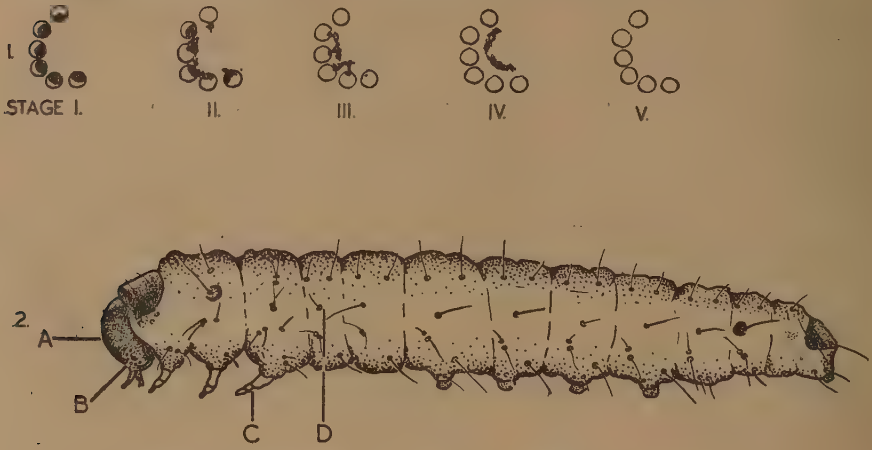


Fig. 3.—Diagram illustrating Dyar's Law.

The Prepupal Stage of *E. elutella*.

The fully fed larvae leave the food and enter a wandering stage. Under warehouse conditions the length of this stage was estimated at 24–56 hours, although some of the marked larvae wandered as long as four weeks (see Richards & Waloff, 1946). At 25°C., 70 per cent. R.H. this stage is generally shorter, and may be omitted by some larvae. After wandering the larvae spin up, and either undergo a prolonged period of quiescence or diapause, or enter the prepupal stage, which precedes pupation. The first intimation of the approach of the prepupal stage is in the gradual withdrawal of the ocellar pigment (see fig. 4). This process appears to correspond with that in *E. kühniella* larvae, described by Kühn and Piepho (1936, 1938), where the transformation of the prepupa is characterised by the loosening of the ocelli from the hypodermis and the secretion of the pupation hormone from the brain.



1. 5 STAGES OF WITHDRAWAL OF OCELLAR PIGMENT.
2. PREPUPA AT STAGE IV.
   A. HEAD BENT VENTRALLY
   B. WHITE OCELLI
   C. APPENDAGES IMMOBILE AND POINTING ANTERIORLY
   D. SOME PIGMENT WITHDRAWN FROM BASES OF SETAE
   BODY SEGMENTS CONTRACTED, BODY STIFFENED AND STRAIGHTENED

Fig. 4.—Diagram of prepupal stage of *Ephestia elutella.*

The life history of *E. elutella* can thus be subdivided into the following stages: (1) Egg. (2) Larva. (*a*) feeding stage accompanied by four or five moults, (*b*) wandering stage of the fully fed larvae, (*c*) spun up stage, which may entail a prolonged period of quiescence or diapause. (3) Prepupal stage, which may follow 2 (*a*), (*b*) or (*c*). (4) Pupa. (5) Moth.

At the beginning of the prepupal stage the larva spins up and becomes less mobile; when stimulated by touch it tends to move backwards. It is convenient to subdivide the prepupal period into the five stages described by Kühn and Piepho. These stages are based on the degree of withdrawal of ocellar pigment (see fig. 4). When stages two and three are reached, the prepupa begins to lose mobility and only wriggles when touched. At this time the hind gut, which hitherto appeared half-full, is evacuated. Bright orange excretions, which rapidly darken and contain uric acid, are given out, and the two posterior abdominal segments become almost transparent. The head is at first drawn into the prothorax, but by stage three is bent ventrally at a sharp angle. The pigment is gradually withdrawn from the sclerotised plates at the bases of setae, and the body grows paler, often milky white. The segments contract, the prepupa stiffens and straightens. At this stage the thoracic appendages are immobile and point stiffly anteriorly.

At stages four and five all mobility is lost, and after complete decoloration of the ocelli the thoracic appendages become curved towards the posterior end and are pressed closely against the body. The whole body finally assumes a pupa-like appearance, and the white pupal skin shines through the loose larval skin. At the last moult the larval skin is first split dorsally in the middle of the abdomen, and the pupa gradually frees itself, splitting the head capsule in the process. The head capsule, and the larval skin, together with the orange excretions are usually found in a bunch at the posterior end of the pupa, inside the cocoon. A freshly moulted pupa is white, but becomes brown in a few hours.

At 25°C., 70 per cent. R.H. the prepupal stage lasts about 5 days. Stages one–four last 3–4 days, four–five a few hours. Pupation usually occurs within 24 hours, and often in under 18 hours after complete decoloration of the ocelli. At lower temperatures the process is slowed down, for instance at the average temperature of 16°C. the interval between stage five and pupation may last 3–5 days.

On ligaturing the head, only fourth and fifth stage prepupae attempt to pupate. The other stages remain as "permanent larvae" and eventually die. Attempts at pupation are imperfect, as the ligatured prepupae are unable to cast off the larval skin and head capsule. Pupation in stage five was very clear, and the shiny pupal skin was formed in all individuals. In stage four attempts at pupation were less clear, but the skin became brown, and patches of shiny pupal skin became apparent, when the larval skin was peeled off.

Summary.

1. A selected stock bred on middlings and not diapausing at 25°C. was used in these experiments. None of the larvae reared on Manitoba wheat diapaused at 25°C., 70 per cent. R.H.; some diapaused at 21°C. and 17°C., 70 per cent. R.H. At these lower temperatures development was slower, more food by weight was eaten, and the moths were heavier.

2. More larval substance by weight was needed to produce a male than a female adult.

3. It was possible to subdivide natural foods into 3 groups: (i) Those on which no larvae diapaused at 25°C., 70 per cent. R.H. (ii) Foods on which a fairly low percentage of larvae diapaused at 25°C. 70 per cent. R.H. (iii) Foods on which most, or all of the larvae diapaused at 25°C., 70 per cent. R.H.; these foods were all characterised by a high content of starch. The possible cause is discussed.

4. Larvae moult 4 or 5 times on Manitoba wheat at 25°C. 70 per cent. R.H. Most of the larvae developing on this food under warehouse conditions could be allocated to 6 instars. At 25°C., 35·5 per cent. R.H. the number of larval instars is increased—one larva died in 12th instar.

5. The growth curves, obtained by measuring head capsules of successive instars roughly follow Dyar's law, although the ratio of increase falls off in the last 2 instars.

6. The prepupal stage in *E. elutella* can be subdivided into five stages, on the basis of withdrawal of ocellar pigment, described in *E. kühniella* by Kühn and Piepho. Only those prepupae which have reached stages four and five attempt to pupate after the head is ligatured, the other stages remain as "permanent larvae".

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I am grateful to Professor J. W. Munro of Imperial College, Professor H. G. Jackson of Birkbeck College and to the Department of Scientific and Industrial Research, under whose auspices this work was carried out.

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References.

Brecher, G. & Wigglesworth, V. B. (1944). The transmission of *Actinomyces rhodnii* Erikson in *Rhodnius prolixus* Stål (Hemiptera) and its influence on the growth of the host.—Parasitology, **35,** pp. 220–224.

Fraenkel, G. & Blewett, M. (1943). The natural foods and the food requirements of several species of stored products insects.—Trans. R. ent. Soc. Lond., **93,** pp. 457–490.

—— & ——. (1946a). The dietetics of the caterpillars of three *Ephestia* species, *E. kuehniella*, *E. elutella*, and *E. cautella*, and of a closely related species, *Plodia interpunctella*.—J. exp. Biol., **22,** pp. 162–171.

—— & ——. (1946b). Linoleic acid, vitamin E and other fat-soluble substances in the nutrition of certain insects (*Ephestia kuehniella*, *E. elutella*, *E. cautella* and *Plodia interpunctella* (Lep.)).—J. exp. Biol., **22,** pp. 172–190.

Goldschmidt, R. (1923). The mechanism and physiology of sex determination. London.

Kühn, A. & Piepho, H. (1936). Ueber die hormonalen Wirkungen bei der Verpuppung der Schmetterlinge.—Nachr. Ges. Wiss. Göttingen (N.F.) **2,** p. 141.

—— & ——. (1938). Die Reaktionen der Hypodermis und der Versonschen Drüsen auf das Verpuppungshormon bei *Ephestia kühniella* Z.—Biol. Zbl., **58,** pp. 13–51.

Richards, O. W. & Thomson, M. A. (1932). A contribution to the study of the genera *Ephestia* Gn. (including *Strymax* Dyar) and *Plodia* Gn. (Lepidoptera Phycitidae) with notes on parasites and larvae.—Trans. ent. Soc. Lond., **80,** pp. 169–250.

—— & Waloff, N. (1946). The study of a population of *Ephestia elutella* Hb. (Lep., Phycitidae) living on bulk grain.—Trans. R. ent. Soc. Lond., **97,** pp. 253–298.

Titschack, E. (1926). Untersuchungen über das Wachstum, den Nahrungsverbrauch und die Eierzeugung. II. *Tineola biselliella* Hum. Gleichzeitig ein Beitrag zur Klärung der Insektenhäutung.—Z. wiss. Zool., **128,** pp. 509–569.

Waloff, N. & Richards, O. W. (1946). Observations on the behaviour of *Ephestia elutella* Hb. (Lep., Phycitidae) breeding on bulk grain.—Trans. R. ent. Soc. Lond., **97,** pp. 299–335.

A NEW SPECIES OF *ERYTHRONEURA* FROM FIJI (HOMOPTERA, JASSIDAE).

By J. W. Evans.

Commonwealth Institute of Entomology.

Erythroneura leveri, sp. n.

Length, 2·9 mm. General coloration pale yellow. *Width of head*, across the eyes, 0·7 mm. *Tegmen* with a brown marking as shown in fig. 1*b*. *Male genitalia* as in fig. 1*a*.

Type ♂ from Suva, Fiji, collected by R. A. Lever 14. viii. 1943, on bean.

According to Mr. Lever, *E. leveri* is a vector of a leaf-crinkle on leaves of Madagascar beans (*Phaseolus lunatus*) and cowpea (*Vigna unguiculata*) in Fiji.

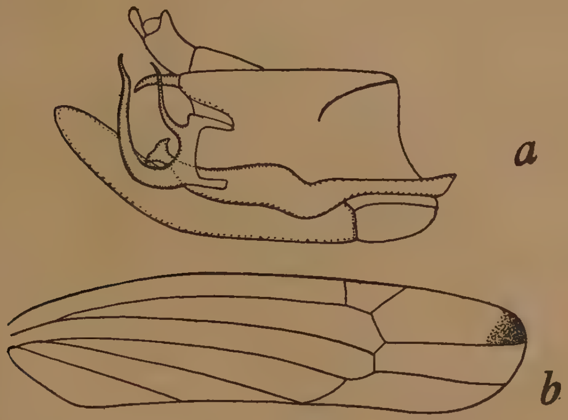


Fig. 1.—(*a*) Male genitalia. (*b*) Tegmen.

THE MOSQUITOS OF THE JEBEL AULIYA RESERVOIR ON THE WHITE NILE.

By D. J. Lewis, M.A.
Medical Entomologist, Stack Medical Research Laboratories, Khartoum.

(Plate XII.)

The Jebel Auliya Reservoir extends some 530 kilometres up the White Nile from the Jebel Auliya Dam, its effect being felt above Renk (Hurst, 1944). The purpose of this great reservoir is to store part of the flood water of the White Nile for use in Egypt during the low river period and is but a part of the scheme, not yet completed, for the training of the Upper Nile. The area under consideration comprises the reservoir, with its thousands of acres of seasonal swamps, and the adjacent land with irrigated areas and rain water pools. Apart from the malarial mosquitos, potential vectors of yellow fever (Lewis, 1947) are also of interest because steamers from the yellow fever area travel northward through the reservoir and meet the railway by which infected individuals might travel to other parts of the country. In addition to the transmission of disease, great discomfort is caused to men and animals by the bites of vast numbers of mosquitos of several species, and, since their existence is mainly due to the reservoir condition, the control of all mosquitos is regarded as a reasonable aim.

The present paper is based mainly on the results of several visits to the reservoir between 1937 and 1946 and it is intended to continue observations, particularly on the bionomics of *Anopheles rufipes*, Gough, and *A. pharoensis*, Theo. Most of the work was done before the reservoir had reached its highest level and even now the conditions of plant growth are not completely stabilised. Lewis (1947) discussed the biting habits of some species. Newhouse (1939) has referred to the possibility of raising the reservoir at some future date.

GENERAL DESCRIPTION OF THE AREA.

It is convenient to consider three sections, the Jebelein section (from Renk to Kosti Bridge), the Kawa section (from Kosti Bridge to Shabasha) and the Geteina section (from Shabasha to Jebel Auliya) (Map 1).

Topography and Climate.

The land slopes gently down to the river and consists of an alluvial clay plain with some sandy areas and a few scattered rocky hills. Average shade temperatures at Dueim, in degrees centigrade, are as follows (Climatological Normals, 1938) :—

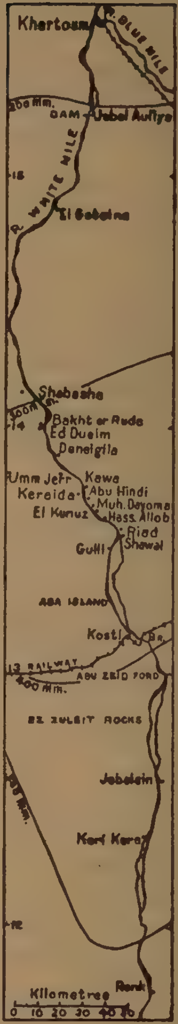
	Annual	December	June
Maximum	37·0	33·7	39·5
Mean	27·3	23·1	30·7
Minimum	20·0	15·5	23·9

The prevalent wind is northerly from October to April and southerly from May to September. At Renk the average annual rainfall is 518 millimetres and at Jebel Auliya 207. Other information on rainfall is given in Map 1 and fig. 1.

Population and Land Vegetation.

Most of the Jebelein section is occupied by Arabs but the boundary between the area of the Arabs and that of the Nilotic negroid people is near Renk. In this southern part of their area the Arabs do not live near the river, mainly owing to the attacks of mosquitos on themselves and Tabanid flies on their cattle, but lead a nomadic life, camping by the river in the dry season and going inland in the rains. There are, therefore, very few villages apart from a few places where steamers call. Much of the country inland is covered with thorn scrub.

In the Kawa and Geteina sections there are many villages and, before the dam began to operate, large quantities of millet were grown on the land left by the falling river. Many of the people have been moved inland, mainly to irrigation pump schemes, to compensate them for the loss of land and collapse of houses due to erosion and seepage, and to reduce the danger of malaria and schistomiasis. Vegetation is sparse in the Kawa and Geteina sections and houses are situated in compact villages surrounded by the wind-swept plain. In the Kawa and Geteina sections most riverain villages were in one of two types of situation with regard to breeding places of mosquitos. In one the village stood near the edge of a swamp in which many *A. pharoensis* bred. In the other the village was on a comparatively steep bank near which were a few inconspicuous pools containing larvae of *A. gambiae*, Giles. At first sight the former, with its great numbers of mosquitos, appeared the more unhealthy. These two typical sites are discussed below in the section on *A. pharoensis*. Many of the people keep cattle and goats.


Khartoum
R. BLUE NILE
200 mm.
DAM
Jebel Auliya
R. WHITE NILE
15
El Geteina
Shebesha
300 mm.
14
Bakht er Ruda
Ed Dueim
Deneigila
Umm Jerr
Kawa
Kereida
Abu Hindi
Muh. Dayoma
El Kunuz
Hass. Allob
Riad
Shawal
Gulli
ABA ISLAND
Kosti
Br.
13 RAILWAY
ABU ZEID FORD
400 mm.
EZ ZULEIT ROCKS
Jebelein
500 mm.
Kerf Kera
12
Renk
Kilometres
0 10 20 30 40 50

Map 1.—The Jebel Auliya Reservoir area, showing places mentioned and isohyets. The river banks are at the natural high level and pump irrigation schemes are omitted.

The River.

The reservoir lies in the part of the river known as the desert reach (Ball, 1939). In this reach the average slope of the river is very low, less than one centimetre per kilometre. The natural annual variation in level is small, and there is very little erosion or deposition of silt. Before the dam was built the yearly Blue Nile flood dammed up the White Nile to a distance of over 150 kilometres above the present dam (Newhouse, 1939). Willcocks and Craig (1913) stated that this ponding extended at least as far as Hillet Abbas, near Kosti, 255 kilometres from the dam site, and described the water as a pulsating lake rather than a river. MacDonald (1920) wrote that it did not extend more than 275 kilometres in a very high flood year. This effect is seen in fig. 2. Lyons (1906) described the flooding of low-lying land, usually till October, and stated that the maximum width of the river reached six to seven kilometres in places.

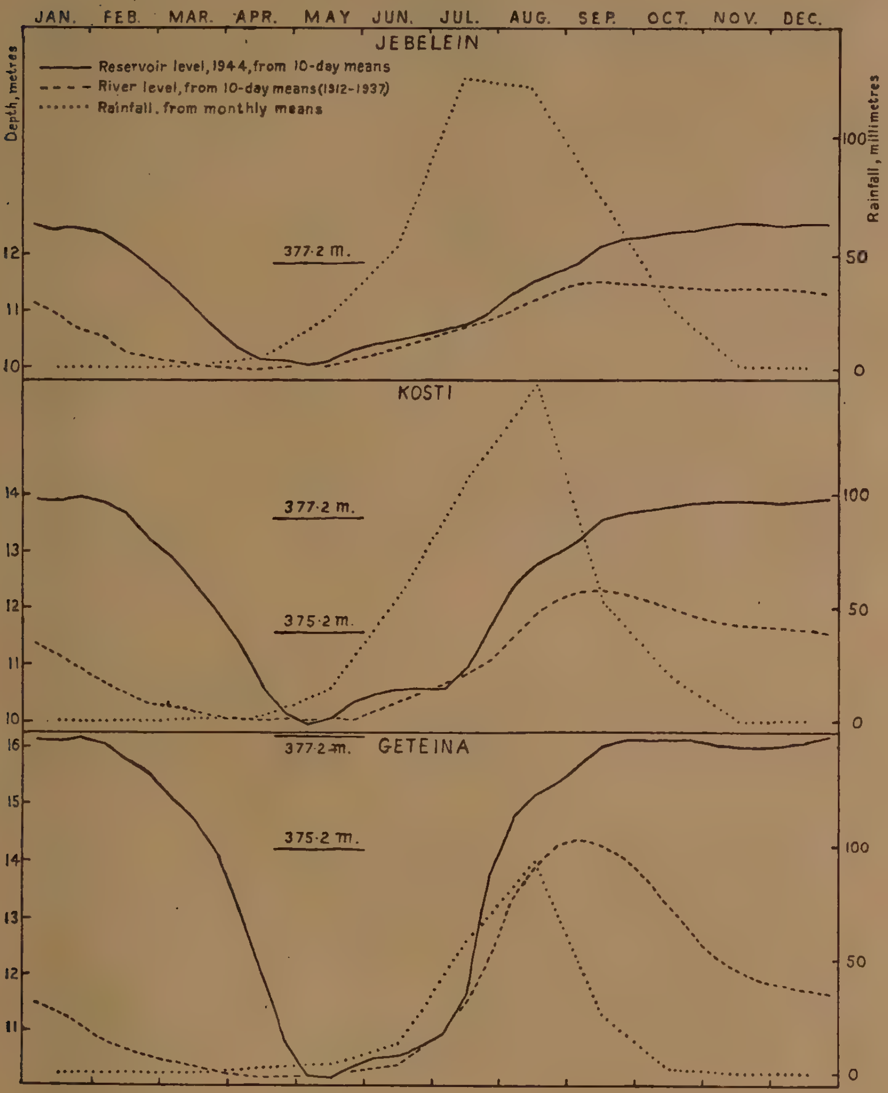


Fig. 1.—Showing mean natural river levels, reservoir levels and rainfall at three places. The Kosti levels are read at Rabak, near the bridge. The zeros of the Jebelein, Rabak and Geteina ganges are 365·38, 363·64 and 361·04 metres above sea level respectively. The full storage level is 377.2 metres and the 375·2 level is indicated to show the approximate lower level of shallow swampy water.

In the Jebelein section a flood plain several hundred metres wide extends for great distances, and bears long narrow furrows parallel to the main channel. Near Kosti Bridge this natural flood plain disappears. In the Kawa and Geteina sections, except opposite Aba Island, the left bank usually slopes gently down to the river and the right is usually comparatively steep and sandy, possibly owing partly to drifting sand which is brought by the prevailing north-east wind in the dry season.

There are many islands in the reservoir area.

The Reservoir.

Water is stored from September to February, the storage level being 377·2 metres above sea level and the storage capacity three thousand million cubic metres (Hurst,

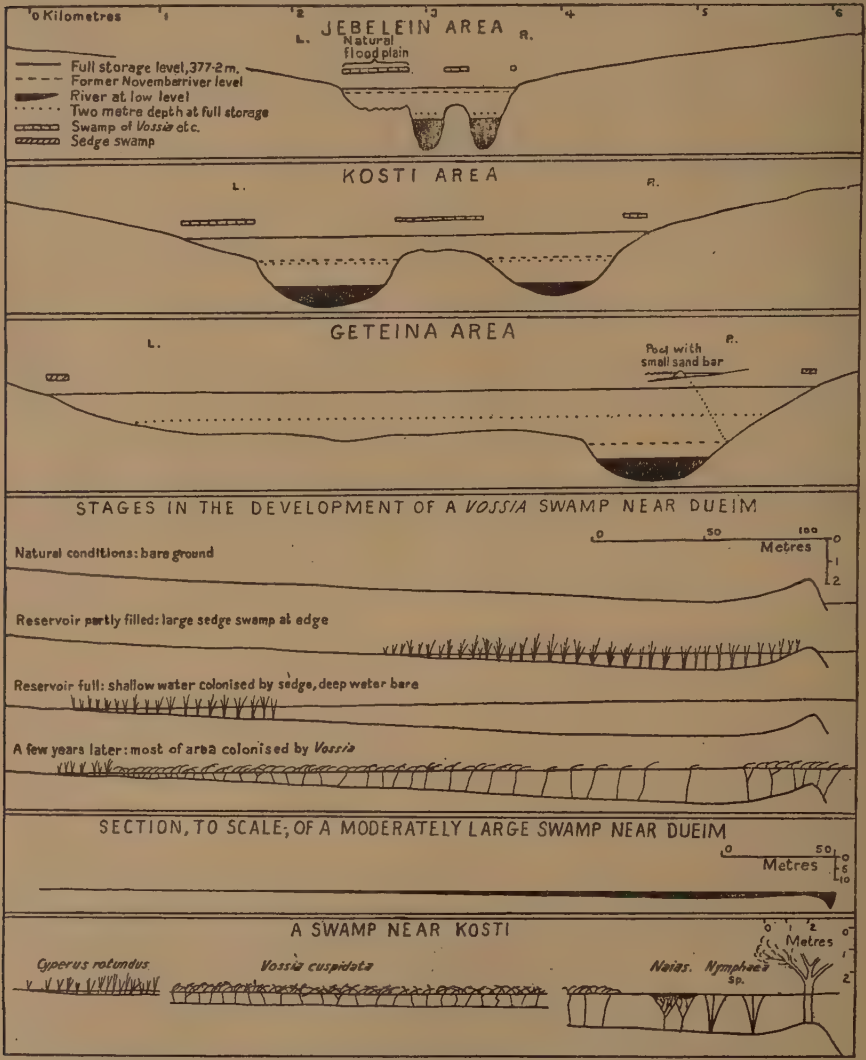

0 Kilometres
1
2
3
4
5
6
JEBELEIN AREA
L.
R.
Natural flood plain
Full storage level, 377·2 m.
Former November river level
River at low level
Two metre depth at full storage
Swamp of *Vossia* etc.
Sedge swamp
KOSTI AREA
L.
R.
GETEINA AREA
L.
R.
Pool with small sand bar
STAGES IN THE DEVELOPMENT OF A *VOSSIA* SWAMP NEAR DUEIM
0
50
100
Metres
0
1
2
Natural conditions: bare ground
Reservoir partly filled: large sedge swamp at edge
Reservoir full: shallow water colonised by sedge, deep water bare
A few years later: most of area colonised by *Vossia*
SECTION, TO SCALE, OF A MODERATELY LARGE SWAMP NEAR DUEIM
0
50
Metres
0
5
10
A SWAMP NEAR KOSTI
0
1
2
Metres
0
1
2
Cyperus rotundus
Vossia cuspidata
Naias.
Nymphaea sp.

Fig. 2.—Sections at various points along the reservoir; all measurements are approximate and the upper three sections are generalised diagrams.

1944). Newhouse (1939) gave the maximum area as 1,318 square kilometres. In the Kawa section the level varies about 40 centimetres during the full storage period because at times the water is banked up by the action of wind and by the curve formed at the junction of the river slope with the flat surface of the reservoir. The "backward curve" is effective between Jebelein and Shawal and its position is influenced by variations in the natural flow of the river. The effect of the wind can be seen west of Umm Jerr when the water flows from north to south through a bridge over a depression flooded by the reservoir. At full reservoir level large areas of shallow water exist. In the Jebelein section these generally comprise the flood plains and islands, with the normal flooding deepened and extended for a long period, and in the Kawa and Geteina sections the shallow areas consist of the islands and of areas which were normally the dry land on each side of the river (fig. 2). In several places, particularly north of Dueim, where there is not much rain water which would be held up, banks have been built to protect certain areas from flooding.

Malaria.

Malaria occurs throughout the area and appears to have increased since the dam was constructed. During a survey made in the winter 1943 and February 1945 the heaviest incidence was among people living within a mile of the river bank or of pump irrigation schemes. In some years rain-water swamps far from the river appear to be responsible for much malaria. Further reference to the disease is made in the section on species of mosquitos.

Present-day Breeding Places and aquatic Vegetation.

The following paragraphs refer chiefly to the area north of Jebelein where breeding places are very scarce from March to July.

The main swamps.

These are considered as being exclusive of the marginal area within a few metres of the shore. The breeding conditions are determined by the prevalence of various aquatic and semi-aquatic plants which affect mosquitos in different ways. Some afford sites for oviposition; some, by reason of their compact structure or growth, afford direct protection in the shape of small spaces in which larvae can escape from predators larger than themselves; some protect breeding areas from wave action; some hinder the growth of plants favourable to larvae.

Many plants afford sites for oviposition but the dense surface-growing species are particularly attractive to *A. rufipes* and *A. pharoensis*.

Plants that afford some protection, particularly when mingled with plant fragments, pieces of gelatinous brown alga or a thick growth of *Spirogyra*, are the semi-aquatic grasses *Vossia cuspidata* Griff. (small variety), *Echinochloa stagnina* Beauv. and *Vetiveria nigritana* Stapf., the sedge *Cyperus rotundus* Linn., flooded land grasses and herbs, and the less abundant plants *Neptunia oleracea*, Lour., *E. pyramidalis* Hitch & Chase, *Ipomoea reptans* Poir. and *Jussieua repens* Linn. Plants which afford greater protection are *Pistia stratiotes* Linn. and *Azolla* sp. and the under-water plants *Naias pectinata* Magnus, *Vallisneria aethiopica* Fenzl., *Spirogyra*, *Ceratophyllum demersum* Linn. and the gelatinous alga. Sedge and other plants sometimes come into this category if they die and fall on the water or are trodden flat by man, cattle or hippopotamus. *N. pectinata* was important in the first years of dam operation when the water was lowered to the level of this plant in the autumn.

Protection from wave action is effected chiefly by larger plants such as the tree *Acacia arabica* Willd., papyrus (*Cyperus papyrus* var. *antiquorum* Clarke), the large variety of *V. cuspidata* known as *umm suf*, *Juncellus alopecuroides* Clarke and various grasses.

The growth of many plants is hindered by *A. arabica*, *V. cuspidata*, *I. reptans* and the broad-leaved *J. repens*.

Many other water plants occur which have little or no effect on breeding conditions of mosquitos.

Three plants deserve further mention owing to the large areas which they cover, the creeping grasses *V. cuspidata* (small variety) and *E. stagnina*, and the sedge *C. rotundus*.

V. cuspidata and *E. stagnina* sometimes grow singly in as much as 3 and 1½ metres of water respectively but often form large rafts, many acres in extent, of dense growth anchored by stems to the bottom, the former species predominating. Such creeping-grass swamps, sometimes thousands of acres in extent and over one kilometre wide are found in water between a few centimetres and 1½ metres deep, that is, in the large areas of the lateral swamps and on submerged islands, in the Jebelein and Kawa sections. North of Shabasha the creeping grasses are found but grow sparsely and in shallower water. These grasses, which, at Kosti at least, survive the dry season in vegetative form, grow to some length in the early rains and, as the water rises, the foliage rises with it. Under the rather unusual conditions of the Sennar reservoir they can flourish in 380 centimetres of water (Andrews, 1945), but in the Jebel Auliya Reservoir they cannot keep pace with the more rapid rise of the water except in shallower places. Grazing by animals in the dry season and, in the north, the low rainfall, operate against the creeping grasses by preventing them from growing to a sufficient length to survive in deep water. Fig. 1 shows how little rain falls before the water rises at Geteina. In shallow water where animals graze the grasses have a thick mass of aquatic roots but little foliage.

C. rotundus is a land plant which can live in water; by means of its tubers and deep root system it can survive a long period of drying (Andrews, 1940). Owing to its erect habit and moderate height it can only live in shallow water, usually less than a metre deep, and its slender leaves can easily be overshadowed by a denser growth of other plants. It grows freely in the shallow water of the Geteina section; it also forms sedge swamps in the Kawa section but here and in the Jebelein section the creeping grasses occupy much of the shallow water.

Of the other plants many species occur in the swamps of the Jebelein section. In the Kawa section the smaller species tend to grow in the sedge swamps where they are not overshadowed by the creeping grasses. *E. pyramidalis*, which grows among the creeping grasses, is common at Jebelein and Kosti and was found as far north as Kawa in 1945. *V. nigritana* is common in the sedge swamps between Kosti and Geteina and probably further afield. In 1945 no papyrus was seen north of Kosti bridge or some kilometres south of it and the most northerly *umm suf* was on Warilat Island just north of Kosti.

South of Jebelein the swamps have been little studied since the population is small. Numerous water plants exist, including papyrus, and the large variety of *V. cuspidata* predominates.

The river margin.

In swampy areas this consists of the edge of the swamp, which is often differentiated from the rest by the presence of foot and hoof marks, the treading down of vegetation and cultivation by simple irrigation methods. In many areas without swamps a common type of breeding place is formed by small creeks blocked by sand or floating debris. Small pools, bare of vegetation, are formed by wave action which builds up low sandbanks that enclose the pools.

Rain-water pools.

These are very variable in number and extent.

Areas irrigated by pumps.

Thousands of acres of cotton and other crops are irrigated from July to February and numerous small pools would form in minor channels if control were relaxed.

Miscellaneous breeding places.

Breeding places of minor importance are wide wells, water-filled boats, tanks, tree-holes and water-jars. Few pools remain after the river has fallen because the natural drainage carries the water away.

A note on the northern section and rainfall.

Several important features of the northern section are due to low rainfall. The steepness of the right bank is probably due partly to drifting sand made possible by the arid conditions. This formation and the scarcity of aquatic vegetation allow the development of small pools by wave action. Comparatively small areas of creeping grass develop. North of Dueim it has been easy to protect large areas from flooding by building banks since little rain water accumulates behind them.

The Effect of the Dam on Breeding Conditions.

The writer did not visit the area till after the dam had begun to operate. Many of its effects can be conjectured, however, from a study of old descriptions and of conditions during the transition period (1937–1942), when the flooding was increased by stages, and by comparing conditions above and below the dam and seeing conditions above the dam at low water.

The Jebelein Section.

This has been altered less than the other two and was infested with mosquitos before the dam was built, owing to the existence of the above-mentioned flood plain. Boulenger (1907) wrote of mosquitos beginning south of Goz Abu Gumah (near Kosti) during a southward journey made in January 1901.

Reservoir conditions have deepened the swamps and lengthened the period of flooding. In the south the effect is very small but in the north new sedge swamps have been formed. Another effect is the reduction in floating papyrus (as at Jebelein) corresponding to that in the Kawa section.

The Kawa Section.

Before the dam began to operate, and when the White Nile was dammed annually to a considerable extent by the Blue Nile, some vegetation must have grown in the lateral swamps. Willcocks and Craig (1913) mentioned flooding without swamp formation 30 kilometres north of Kosti Bridge. Whatever sedge swamps did exist during the flooding period have been greatly increased, creeping grass swamps have developed, and the period of flooding has been lengthened.

An important feature was caused by the masses of papyrus and *umm suf* (*V. cuspidata*) which annually drifted down on the flood from the great swamps. In the ponded part of the river when the current was reduced this "sadd"* vegetation became stranded in two or three metres of water. In the shallow water between the papyrus and the banks a swampy strip was formed of grasses and other plants, some of them *Pistia* and *I. reptans* brought down with the papyrus. Papyrus, stranded along the edges of swamps, protected them from wave action and intensified the breeding of mosquitos. A strip of stranded papyrus near Kosti is shown in a photograph by Vageler and Altern (1932). The sadd was regarded by the inhabitants as a source of mosquitos, probably owing to the papyrus-protected swamps. Although the papyrus plants alone probably did not bring the larvae, since the plants are often surrounded by small fish which can penetrate among the rhizomes, some larvae might have been brought down by the large floating masses such as those on which men could walk.

In 1937 there were dense masses of papyrus at Kosti bridge and for great distances down to and beyond Shawal the banks were lined with it (Pl. XII, fig. 1). The most northerly piece was seen opposite Dueim where *umm suf* also had stranded. Balfour (1904) stated that sadd was rarely encountered north of Dueim. Casati (1891) mentioned vast tracts of *Vossia* and papyrus near Kawa in July 1880. At Kosti in 1937 booms were being used to ward off floating masses of sadd from the quay and a steam tug was being used in the removal of stranded masses. Floating sadd passed Kosti in varying quantities each year and in 1901 (Boulenger, 1907) was not seen as far north as Kosti. That it has not always reached this latitude is shown by Schweinfurth's (1874) record of sailing up the Nile in 1869 and getting his first sight of papyrus west of the Bahr el Zeraf mouth. Other authors mention various northern limits and it appears that the amount of sadd drifting downstream has varied from time to time according to the height of the river and the condition of channels through the Sadd.

Baker (1866), who sailed up the White Nile in December 1862, alluded to the inundation of land on each side of the river and to the descent of vegetation from upstream. After referring to the area flooded during the rains near Dueim he writes of the acacia forest between Dueim and Jebelein, probably near Kosti: " . . . on a closer approach the forest proves to be a desolate swamp, completely overflowed; a mass of fallen dead trees protruding from the stagnant waters, a solitary crane perched here and there upon the rotten boughs; floating water plants massed together, and forming green swimming islands, hitched generally among the sunken trees and branches; sometimes slowly descending with the sluggish stream, bearing, spectre-like, storks thus voyaging on nature's rafts from lands unknown. It is a fever-stricken wilderness—the current not exceeding a quarter of a mile per hour—the water coloured like an English horse-pond; a heaven for mosquitoes and a damp hell for man; fortunately, this being the cold season, the winged plagues are absent."

It was expected by some that the creation of vast swamps would allow floating papyrus to approach Khartoum and to choke the reservoir. In fact the opposite has happened as was foretold by Sir Murdoch MacDonald (1920). The floating masses reach the ponded water further upstream than before and tend to strand between Renk and Jebelein. As the water rose higher each year less papyrus drifted past Kosti and in October 1945 and 1946 not a single plant was seen near Kosti bridge. Although 1945 was a year of low flood and little papyrus came down the river, nevertheless the "papyrus-protected" swamps of the Kawa section are probably a thing of the past. This great alteration in the aquatic flora appears to extend at least from Jebelein to Kawa.

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* "Sadd" means a block in Arabic and was applied to the masses of vegetation which blocked the Upper Nile in the swamp or Sadd region.

Altogether, the reservoir conditions have had the effect of creating extensive grass and sedge swamps by flooding, have filled small watercourses by maintaining a constant high level, have increased the effect of the waves in building up the small sand bars which enclose pools and have brought to an end the papyrus-protected swamps. Many acacia trees are likely to be killed and experimental plantations are being made which may change breeding conditions.

During the transitional period a striking succession of plant colonisations occurred in parts of the Kereida-Shabasha swampy areas. At first great stretches became overgrown with *C. rotundus* as the lowest land was flooded. This next became too deep for sedge which grew only in a belt along the newer shore line. Much of the deeper water was then colonised by *E. stagnina* and *V. cuspidata*. The three stages were thus : (1) large sedge swamps, (2) small sedge swamps, and (3) small sedge swamps with large grass swamps (fig. 2). *V. nigritana*, a common swamp grass in the southern Sudan, was first seen in the reservoir at Kereida in 1944.

The Geteina Section.

Before the dam was built some temporary swamps may have been formed during the damming of the White Nile by the Blue. The changes have been essentially the same as in the Kawa section except that the sedge swamps are smaller ; there are no large creeping grass swamps, and papyrus never existed.

Predacious Insects and Fish.

There are some large areas of swamp where plants do not grow compactly and larvae are scarce, probably owing to the action of predators. Carnivorous insects are common and among them the Belostomid Hemipteron, *Sphaerodema nepoides*, F., is conspicuous.

Barbus stygmatopygus, Boulenger.

This small cyprinid is common but its habits are unknown.

Micralestes acutidens, Peters.

This small, very active, characinid, allied to the ferocious tiger fish, commonly cruises near swamp plants. It does not appear to penetrate vegetation but perhaps eats mosquito larvae which leave the protective plants as they have been seen to do in water where fish are few.

Aplocheilichthys loati, Boulenger.

Pekkola (1919), writing of this top-minnow and *E. marnoi* and *E. bifasciatus* in the Upper White Nile, stated that they swam near the surface in shoals of about 4 to 50, that they fed exclusively on insects which they caught on the surface, and that they were observed beginning to spawn in August. Boulenger (1907), writing of *A. schoelleri*, which is very closely related to *A. loati*, stated that it was oviparous and hung its eggs in bunches to the twigs of plants by means of small threads. *A. loati* is very common in the reservoir. A specimen taken near Kawa was found to contain the remains of 38 insects, including three second-stage larvae of *Culex poicilipes*, Theo., and one first-stage Anopheline, but the fact that mosquito larvae are often found in the presence of this fish shows that it only exercises partial control over them. This species cruises in shoals at the surface in spaces among swamp vegetation and will cross open spaces. It passes into main irrigation canals through the pumps.

A. loati was common at Shawal on 19.xii.37 and is often abundant in the Jebelein section. In view of its absence on the Blue Nile it seems unlikely that this swamp-living top minnow can survive during the low water period in the main river with its many large carnivorous species, and the scarcity or absence of the fish in the Geteina section might be explained by supposing that it only lives permanently far to the south in the Jebelein section. In this area (near the Abu Zeid Ford) residual pools were seen in the swamps in April 1945 (although no fish were found) and other possible dry season refuges were patches of *Vossia cuspidata* anchored in lateral channels and near islands. *A. loati* was seen at Kosti on 8.vii.39 and was abundant at Gulli and Riad in September 1939. It has been seen as far north as Dueim (29.i.39) and Bakht er Ruda (11.ix.39). At Kereida none could be found on 22.i.39 or 12.ix.39; one was seen there on 18.x.39 but the scarcity of this species in the area, in contrast to Kosti, was surprising. At Bakht er Ruda on 29.i.39 no *A. loati* and few predacious insects were seen in sedge swamps and mosquito larvae were very numerous. At Dueim on 16.xii.46, however, the fish was abundant.

Repeated attempts to maintain stocks of *A. loati* and *Epiplatys marnoi* in the ten-square-metre pools at Wad Medani were unsuccessful although such pools can support large numbers of *Gambusia holbrooki* and *Lebistes reticulatus*. In March 1940, 319 *A. loati* and 87 *E. mernoi* were placed in two pools but a year later the numbers were only 125 and 7 respectively.

Epiplatys marnoi Steindachner.

This brown diagonally-striped cyprinodontid is less common than *A. loati*. It appears to spend more time in vegetation and to leave it less readily and does not usually swim in shoals. Many of this species were seen with numerous Anopheline larvae among *Potamogeton* sp. in Lake No in the Sadd Area. Nine were dissected and no mosquito larvae found in them, evidently owing to the protection afforded by plant fragments. Material was then collected from one square metre of the water surface and left in a basin for 25 minutes. In seven fish then dissected one or more Anopheline larvae, evidently exposed by the disturbance, were found in each. *E. marnoi*, when kept in glass jars, has been found to breed in April. It is often abundant in the Jebelein section and has been found at Kosti on 15.vii.39 and often in later months. An allied species, *E. bifasciatus* Stdr. has been found at Kosti and Gulli.

Haplochromis multicolor, Schoeller.

This small cichlid, which holds its young in its mouth, lives in thick vegetation, usually below the surface. It multiplies rapidly in captivity and feeds readily on larvae of *Aëdes aegypti* but often fails to eat those of *Culex univittatus* and does not prevent Anophelines breeding in stock ponds. It was found breeding at Abu Zeid on 22.xii.38 and is sometimes seen at Kosti.

Tilapia spp.

The young soon grow too large to eat mosquito larvae, if they do so at all. These fish eat algae attached to water plants and probably in so doing expose mosquito larvae to the attacks of top minnows. These and other moderately large fish in the swamps probably eat top minnow.

THE MOSQUITOS.

The following 43 forms of Culicidae have been found in the area :—

Chaoborus ceratopogones, Theo.

Anopheles
(*Anopheles*)
coustani var. *ziemanni*, Grünb.
(*Myzomyia*)
nili, Theo.
funestus, Theo.
wellcomei, Theo.
gambiae, Giles
rufipes, Gough
pharoensis, Theo.
squamosus, Theo.

Uranotaenia
balfouri, Theo.
mashonaensis, Theo.

Aëdomyia
africana, N.-L.

Ficalbia
(*Mimomyia*)
splendens, Theo.
hispida, Theo.
lacustris, Edw.
mimomyiaformis, Newst.
plumosa, Theo.
(*Etorleptiomyia*)
mediolineata, Theo.
(*Ingramia*)
uniformis, Theo.

Taeniorhynchus
(*Coquillettidia*)
metallicus, Theo.
chrysosoma, Edw.
(*Mansonioides*)
africanus, Theo.
uniformis, Theo.

Aëdes
(*Stegomyia*)
aegypti, L.
metallicus, Edw.
unilineatus, Theo.
vittatus, Big.

(*Aëdimorphus*)
arabiensis, Theo.
ochraceus, Theo.

(*Banksinella*)
circumluteolus, Theo.

Culex
(*Lutzia*)
tigripes, Grp.

(*Culiciomyia*)
nebulosus, Theo.

(*Mochthogenes*)
simpliforceps, Edw.

(*Culex*)
poicilipes, Theo.
bitaeniorhynchus, Giles
ethiopicus, Edw.
univittatus, Theo.
,, var. *neavei*, Theo.
pipiens, L.
,, subsp. *molestus*, Forsk.
antennatus, Beck.
decens, Theo.
perfuscus, Theo.

TABLE I.

Mosquito larvae collected in the Kosti swamps, exclusive of the margins, between July 1939 and February 1941 (percentages in italics).

A. coustani var. *ziemanni*[1]	32	*5*	*U. balfouri*	61	*2*
A. funestus	3	*0·4*	*Aë. africana* ...	23	*1*
A. wellcomei	179	*26*	*F. splendens* ...	1,387	*41*
A. gambiae	20	*3*	*F. lacustris* ...	2	*0·1*
A. rufipes	125	*19*	*F. mimomyiaformis*	84	*3*
A. pharoensis ... ... *A. squamosus* (very few) ...	318	*41*	*F. mediolineata* ...	5	*0·2*
			C. poicilipes ...	1,412	*42*
			C. bitaeniorhynchus or *ethiopicus*	71	*2*
			C. univittatus, etc.[2]	351	*10*
	677			3,396	

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[1] Probably none of type form.

[2] Var. *neavei*, *C. decens* and *C. antennatus*.

TABLE II.

Larvae and pupae collected in the Kosti swamp (except the margin) between November 1942 and February 1943.

Square metres examined ...	5	2	2	2	1	1	1	2	1	1	1	1	1	0·5	1	22·5
Species of third- and fourth-stage larvae and pupae	*C. rotundus*, open	*C. rotundus*, normal	*E. stagnina* and *V. cuspidata*	*C. rotundus*, trodden	*C. rotundus*, recumbent	*C. rotundus*, semi-recumbent	*C. rotundus*, thick	*Vallisneria* sp.	Mixed submerged plants	*Spirogyra*	*I. reptans*	*N. oleracea*	*E. pyramidalis*	*P. stratiotes*	*Azolla* and some *P. stratiotes*	Totals
A. coustani var. *ziemanni* ...	—	—	—	—	—	3	6	—	—	—	—	—	—	—	—	9
A. wellcomei	—	5	—	—	1	123	25	—	—	—	—	—	—	—	—	154
A. gambiae	—	—	—	5	—	—	—	—	—	—	—	—	—	—	—	5
A. rufipes	—	2	—	26	4	—	—	48	116	62	1	—	—	—	—	259
A. pharoensis	—	2	1	21	28	—	15	18	90	9	2	2	2	2	1	193
U. balfouri	—	—	—	4	8	4	—	—	—	—	—	—	—	—	—	16
U. mashonaensis	—	—	—	2	3	3	—	—	—	—	—	—	—	—	—	8
F. splendens	—	—	2	—	—	—	—	—	1	1	8	7	—	59	70	148
F. mimomyiaformis	—	—	—	1	6	1	—	—	—	—	1	3	—	—	2	14
F. uniformis	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	3
C. simpliciforceps	—	—	—	—	—	9	—	—	—	—	—	—	—	—	—	9
C. poicilipes	—	—	1	17	13	1	2	7	70	16	—	1	2	—	—	130
C. ethiopicus or *bitaeniorhynchus*	—	—	—	22	—	—	—	2	—	—	—	—	—	—	—	24
C. decens	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	2
Total (all stages)	1	17	11	452	438	536	83	223	434	279	21	33	10	80	89	—
Per cent. beyond second-stage	—	53	36	22	17	27	58	34	64	32	57	39	40	76	85	—
Fourth-stage and pupae ...	0	5	3	21	34	68	29	19	84	14	6	6	1	42	50	—
Anophelini	1	17	3	289	297	373	81	183	329	242	4	13	8	4	1	—
Culicini	0	0	8	163	141	163	2	40	105	37	17	20	2	76	88	—

TABLE III.

Some collections of adult mosquitos (percentages in italics).

Species	Biting man outdoors in evening								Outside house screening in evening		In house in evening	In houses by day				
	Jebelein 8.i.43 ½ hour		Shabak 26.i.39		Kosti, xi.42, i.43, three half-hour catches		Kereida, 18.x.39		Kosti, house far from river 3 evenings viii.ix.39	Bakht er Ruda 20.x.39, 1 house	Bakht er Ruda 21.x.39, house far from river	Jebelein, 20.i.43, one house	Kosti, vii.39–ii.41, various collections of Anophelines		Kosti, xi.42–ii.43, 48 houses near river	
A. coustani var. *ziemanni*	77	*32*	12	—	61	*7*	3	*1*	4	—	—	—	—	—	1	*0·4*
A. funestus	—	—	—	—	—	—	—	—	—	—	—	34	22	*2*	1	*0·4*
A. wellcomei	2	*1*	63	—	119	*10*	—	—	1	—	—	—	11	*1*	—	—
A. gambiae	—	—	—	—	—	—	—	—	8	28	11	—	1140	*85*	111	*42*
A. rufipes	—	—	—	—	—	—	—	—	—	1	33	—	129	*10*	144	*55*
A. pharoensis	17	*7*	59	—	434	*47*	289	*99*	74	59	5	—	35	*3*	6	*2*
T. uniformis	98	*40*	13	—	2	*0·2*	—	—	6	—	—	—	—	—	—	—
C. poicilipes	3	*1*	15	—	287	*31*	—	—	7	—	—	—	—	—	—	—
C. antennatus	47	*19*	11	—	28	*3*	—	—	—	—	—	—	—	—	—	—
Other species	4[1]	*2*	1[2]	—	—	—	—	—	18[3]	1[4]	1[4]	—	2[4]	*0·1*	—	—
Total	248		174		931		292		118	89	50	34	1339		263	

[1] 1 *A. nili*, 3 *T. africanus*.

[2] 1 *A. nili*.

[3] 2 *Aëdom. africana*, 1 *F. splendens*, 1 *Aëdes metallicus*, 14 *Culex* spp.

[4] 1 *A. squamosus*.

TABLE IV.

Mosquitos collected in seven 15-minute periods near Kosti on 10.xi.42 by one person standing at the edge of the swamps. The first *C. poicilipes* was caught after 25 minutes and the first *A. pharoensis* after 27 minutes ; the period of most active biting appeared to be between 28 and 50 minutes after sunset.

Minutes after sunset	0–15	15–30	30–45	45–60	60–75	75–90	90–105	Total
A.c. var. *ziemanni*	—	2	1	4	—	—	—	7
A. pharoensis ...	—	9	113	34	23	7	9	195
C. poicilipes ...	—	6	51	29	19	10	3	118
C. antennatus ...	—	1	2	—	—	—	—	3
Total ...	0	18	167	67	42	17	12	323

Although a few species occur in vast numbers the total is only about a third of those known in the Sudan. The reason is evidently that the main breeding places, although large, are only suitable for swamp-breeding species and that the area is too far north for many of these to flourish. In addition, much of the swamp has to be recolonised each year.

The Jebelein Section.

A. coustani var. *ziemanni* is common. Table III shows the number of this and other species caught at Jebelein (named after its two rocky hills) a place noteworthy as being the most northerly point on the river at which *A. funestus* is common. This species has also been found at Renk and Keri Kera and probably occurs over a wide area south of Jebelein. Most of the species occur in the Jebelein section. *F. hispida*, *T. africanus* and *C. perfuscus* are known as far north as Jebelein and many species extend into the Kawa and Geteina sections. *T. metallicus* has been recorded from Renk and probably occurs further north.

The Kawa Section.

Some results of collecting mosquitos under various conditions in this area are summarised in Tables I to IV. The square metre areas in which larvae were collected were approximately judged by measured sweeps of a large net of known diameter, the tenacity of the vegetation often precluding a more accurate method.

In the Dueim area most of the less common species found near Kosti are unknown and *A. c.* var. *ziemanni* and *A. wellcomei* are generally few. Catches at Kereida and Bakht er Ruda (Table III) show a comparatively small number of species.

In the Kawa section are the most northerly known limits, in the reservoir area, of several swamp-breeding species. These are *A. nili*, *A. funestus*, *U. balfouri*, *U. mashonaensis*, *Aëdomyia africana*, *F. lacustris*, *F. mimomyiaformis*, *F. plumosa*, *F. mediolineata*, *F. uniformis*, *C. ethiopicus* and *C. decens* at Kosti, *C. bitaeniorhynchus* at Gulli, *T. chrysosoma* at Shawal, *A. wellcomei* at Kereida and *F. splendens* and *C. antennatus* at Dueim.

Larvae.

The principal species breeding in the swamp area at Kosti are *A. wellcomei*, *A. rufipes*, *A. pharoensis*, *F. splendens* and *C. poicilipes* ; in the winter they constituted 91 per cent. of the larvae identified.

In Table II the figures for fourth-stage larvae and pupae in known (though small) areas give an idea of the probable amount of breeding in different types of vegetation at Kosti. In the main sedge and floating grass swamps the number varied from less than one to about two per square metre, and in the patches of submerged plants and damaged sedge from 10 to 68. The number was high in *Pistia* and *Azolla* but most of the larvae were *F. splendens*.

Although the areas sampled were small, the percentage of individuals beyond the second stage appears worthy of note. It is low in some of the plants with the highest number of larvae. This would be surprising if the number of larvae depended on protection from predators. The figure might be explained by supposing that the plants affording greatest protection also afford the best sites for oviposition and that some of the crowded older larvae disperse to neighbouring areas.

The record of *A. gambiae* in Table II calls for comment. This species does not normally breed in the swamps away from the margin except occasionally in tracks near the shore. In the instance noted the track was made by people wading through the sedge and the larvae were found 20 metres from the shore. Such breeding places are an extremely small proportion of the swamps and are easily detected.

A. wellcomei breeds principally in thick sedge, here and at Jebelein.

As may be seen from the tables, several Culicines have only been found rarely and some only once. Possibly these species spread occasionally from the south.

The swamp margins, rain water pools and occasionally pools in irrigated land are the main breeding places of *A. gambiae* and *C. univittatus*. In the swamp margins *A. gambiae* breeds chiefly in places where the vegetation is disturbed by human activity and where land vegetation has been flooded by the river. The small flooded creeks, already mentioned, are important breeding places of *A. gambiae*. In the cotton growing area of the Gezira Irrigation Scheme it has been found that *A. gambiae* tends to breed mainly near villages. This tendency probably exists also in the White Nile pump schemes and may account for the fact that many breeding places far from villages, apparently suitable in other respects, are devoid of larvae.

The common man-biting Anophelines are *A. gambiae* (with a relatively very small breeding area), *A. rufipes* and *A. pharoensis*. *A. wellcomei* has a restricted breeding area and is probably far less numerous than the two latter species. *A. rufipes* breeds in large numbers in some swamps.

Adults.

Near Kosti, on most evenings during the breeding season, great numbers of mosquitos fly from swamps towards the town. Catches (Tables III and IV) show that most of those which bite man near swamps at this time are *A. pharoensis*. So many mosquitos take part in these flights that a half-hour catch on 6.i.43 by one person yielded 400 mosquitos. Many of these mosquitos reach the town, as is shown by the fact that many *A. pharoensis* can be caught among the houses soon after the flight begins. In the morning, however, only small numbers of this species can be found by careful searching. *A. rufipes*, on the other hand, is common in the houses and sometimes outnumbers *A. gambiae*. It is less endophilic than *A. gambiae* however ; at Kosti, in spite of its vast breeding area, it is little more numerous in houses than *gambiae*, which has a very small breeding area.

Two catches were made at Kosti in December 1945 which give some information on the feeding habits of some species. The mosquitos were caught near the swamp on a man wearing shoes, trousers and shirt and on a calf five metres from him, the total time being about 45 minutes. The figures are as follows (percentages are in italics). The catch on the calf might have been larger if the animal had not been restive.

	Man		Calf	
A. coustani var. *ziemanni* ...	2	*0·9*	3	*2*
A. wellcomei	53	*23*	26	*21*
A. pharoensis	106	*47*	53	*44*
T. uniformis	3	*1*	10	*8*
C. poicilipes	57	*25*	7	*6*
C. univittatus	1	*0·4*	—	—
C. antennatus	4	*2*	22	*18*
	226		121	

A striking feature of collections of adult Anophelines hitherto made in this area is the high proportion of females in the later stage of ovarian development. Thus of 171 females of *A. gambiae* and *A. rufipes* collected at Kosti in January and February 1943, 98 (57 per cent.) were in stage five. Of the *A. rufipes*, 82 were collected in February and 68 (83 per cent.) of these were in stage five. Christophers and others (1941) found that, even though many breeding places were dry, an accumulation of females in the last stages of gravidity was rare and it appeared that even when there were no breeding places Anophelines managed to get rid of their eggs when they became mature. At Kosti breeding was continuous and there were breeding places near the town.

The high proportion of "ripe" females does not appear to be a peculiarity of the reservoir area since it has been noted in *A. gambiae* and *A. pharoensis* (with its very different habits) at Sennar and near Wad Medani where the Anophelines collected in villages in October and November 1946 were as follows :—

	Males	Females	Ovarian stages of those dissected I–III	IV, V
A. gambiae	354	2,677	859	1,028
A. rufipes	272	351	112	160
A. pharoensis	0	1	1	0

A. rufipes and *A. pharoensis* are further discussed below.

The Geteina Section.

A. gambiae, *A. rufipes*, *A. pharoensis*, *C. poicilipes* and *C. univittatus* are the common species. *A. gambiae* breeds in the pools formed by wave action.

The Effect of the Dam.

There are some old records of mosquitos which may be mentioned although they give little or no indication of changes in the mosquito fauna. Balfour (1904, 1906) recorded some swamp-breeding species at Renk, Jebelein and near the site of Kosti Bridge. Mr. W. Ruttledge has recorded (in Government files) *A. funestus* at Kosti in September 1935, *A. pharoensis* and *T. uniformis* at Kosti in October 1933, and *A. wellcomei* at Jebelein in September 1933.

There is an acacia plantation near Khartoum on the White Nile where *A. pharoensis* breeds in years when enough rain falls for herbage to grow before the river rises. Similar conditions probably arose in the Geteina section before the dam was built.

In the Jebelein Section the dam has had the effect of prolonging the breeding season.

In the Kawa and Geteina sections the dam has evidently vastly increased the numbers of *A. pharoensis* and other species in the swamps and greatly lengthened the breeding season. On the other hand, by preventing the formation of " papyrus-protected " swamps in the Kosti-Kawa area, it has reduced breeding near many villages situated on relatively steep banks. *A. gambiae* sometimes bred on the shore edge of these swampy strips. By increasing the formation of pools protected by sand bars the reservoir has apparently increased the breeding of *A. gambiae.* In general one may conclude that the reservoir conditions have prolonged the mosquito breeding season, increased the number of *A. gambiae*, and vastly increased those of *A. rufipes*, *A. pharoensis* and other swamp breeders.

Some residents at Kosti considered that mosquitos were fewer in 1945 and 1946 than before and some at Dueim reported apparent reduction in 1946. If this is true it may have been due to the increase of *Vossia* grass over sedge.

Observations on certain Species.

Chaoborus ceratopogones, Theo.

An adult was found at Kosti in January 1944.

Anopheles wellcomei.

At Kosti this species appears to breed entirely in sedge swamps. Its larvae are easily collected with a net in spite of its climbing habit noted by Lewis (1939). Although a common species at Kosti, *A. wellcomei* is rarely found in houses and seems unlikely to be of any importance as a malaria carrier. On 27.i.39 a female was seen biting at 08.30 hours in a swamp south of Kosti.

A. gambiae.

The breeding habits and adult behaviour of this well-known species are as would be expected. In the swamps its larvae are only found near the shore, especially in disturbed vegetation. It breeds near and far from villages. It is probably the main vector of malaria. Reference to this is made in the section on *A. pharoensis.* On rare occasions single females have been found biting at dusk far from villages.

A. rufipes.

This is the only species, apart from *A. gambiae*, which is commonly found in houses between Kosti and Jebel Auliya. If, as is probable, it transmits malaria on the White Nile, its control constitutes a considerable problem as its breeding places are far more extensive than those of *A. gambiae.* It has been found at many places between Keri Kera and Jebel Auliya and, although near the northern limit of its distribution in the Sudan, evidently occurs all along the reservoir. While considering this species it is of interest to note that it is abundant at Rahad in Kordofan Province during the rainy season. Anophelines collected in buildings there included 116 of *A. rufipes* and 4 of *A. gambiae.* In the neighbouring inland swamp 108 larvae of *A. rufipes* were obtained and 11 of other Anophelines but none of *A. gambiae.* At El Amira in the same Province 22 larvae of *A. rufipes* and one of *A. gambiae* were found in a grassy pool and at Ghabsha and Sherkeila *A. rufipes* was commoner in houses than *A. gambiae.*

A. rufipes evidently breeds most profusely in sub-surface vegetation, where it sometimes outnumbers *A. pharoensis*, and in recumbent *C. rotundus*. These breeding places appear to be preferred sites for oviposition as well as places where the larvae are protected from predators. It occurs among erect sedge but not always; at Bakht er Ruda, on 29.i.39, 252 Anopheline larvae from the large sedge swamp were examined and all were *A. pharoensis*. *A. rufipes* does not appear to breed much in the floating-grass swamps unless the foliage is eaten off by cattle. At Wad Medani its larvae have been found in a seepage pool in the dry season.

In the Northern Sudan *A. rufipes* has been found biting man, cattle and sheep.

The nocturnal behaviour differs from that of most species. It has never been taken in collections of mosquitos biting at dusk near breeding places in the reservoir area nor in any such catches of more than 3,500 mosquitos in various parts of the country. At this time of the evening, however, it has, at three places, been found biting several hundred metres from the breeding place. At Kosti a few were found biting, with a few of *A. gambiae* and other species, far from houses and over 500 metres from the edge of the swamp where many mosquitos were biting at the same time. Near Hawata, in Kassala, Province, a few females of *A. rufipes* and *A. gambiae* were seen biting man at dusk in an open sesame field 500 metres from the nearest breeding place. At Rahad the writer collected mosquitos near a swamp and immediately returned to the town to find *A. rufipes* biting a calf and a sheep out of doors. It may be that these more or less endophilous species do not normally bite unless they are in or near houses and that many, failing to find houses, are stranded in open country at dawn and rest in soil cracks, biting if any person happens to approach the spot on a subsequent night. *A. rufipes* has seldom been found in the evening in the screening of houses but may not infrequently be seen biting in houses in the evening as at Bakht er Ruda (Table III).

The prevalence of *A. rufipes* and *A. gambiae* in houses by day at Kosti and elsewhere and the large proportion of "ripe" females have been noted above. At Rahab, adults of *A. rufipes* were found resting in a hedge near houses. The figures for the Wad Medani area suggest that males of *A. rufipes* tend to rest in houses more than those of *A. gambiae*.

It is evident that *A. rufipes* should be classed as a house-haunting species in the reservoir area since many bite in houses by night and many remain in houses by day. It also resembles the endophilous *A. gambiae* in its absence from catches near breeding places about dusk and occurrence out of doors far from water at this time. It is, however, difficult to understand why, if it is an endophilous species more are not found in houses by day. Until more is known of this species it seems advisable to regard *A. rufipes* as a less important vector of malaria than *A. gambiae*.

A. pharoensis.

The bionomics and possible relation to malaria of this species are of great interest because it occurs in millions in the reservoir area, breeds in large numbers in the Sennar Reservoir and flooded Blue Nile basins and is common in many other parts of the Sudan.

In the Northern Sudan it breeds in almost any swamp or pool overgrown with vegetation.

It has been seen how this species will bite man in great numbers near swamps for a limited period. Dusk catches a few hundred metres inland do not yield great numbers. It has been noticed after this period when many have been biting people on steamers that large numbers of females, many of them unfed, will rest for some time on the deck structures in the vicinity. Perhaps after this they bite at various times during the night as Haddow (1942) has found in Kenya. The preference for resting by day in vegetation rather than houses is amply illustrated at Kosti and other places where, after the immense nightly invasions, very few can be found in houses although some can almost invariably be seen in the small sheaves of millet straw which are stacked against some of the houses. At Sennar, where there is much *E. stagnina* and many of the huts have straw porches, many adults can be found in these types of "foliage". When grass grows near the river bank at Kosti *A. pharoensis* can be found in it. The disappearance of great numbers of this species from the town during the night or morning is remarkable and it is supposed that they leave the town to seek shelter in vegetation and that many die in the dry country beyond the town.

Unusual records are of two females biting man at 13·15 hours in strong sunlight in a swamp at Kereida in January 1939, and of a female biting at 09.00 hours in a screened cabin of a steamer in the Sadd.

From the information collected by Evans (1938) it appears probable that this species is not an important vector of malaria in most parts of Africa but may be important in some. Garnham (1945) has discussed the strange differences in capacity to carry malaria exhibited by this species. Evans refers to Symes's observations on its preference for feeding on cattle rather than people, and Haddow (1942) has observed its zoophilism. Although Henderson (1932) stated that it was probably one of the chief vectors in the Upper Nile Province he gave no particular reasons for this conclusion.

In the reservoir area we know that *A. pharoensis* readily bites man and animals, that it normally leaves houses before daytime, and that many females rest in vegetation and very few in houses in relation to the numbers breeding. There is little or no vegetation near most houses during most of the breeding season, and the villages are not surrounded by breeding places but normally separated from the one main breeding place by a stretch of barren wind-swept ground. It is unlikely therefore that there is a close association between people and females of *A. pharoensis* resting in vegetation.

It has been assumed for the present, as a working hypothesis, that the species is of little importance as a vector of malaria in the area in comparison to *A. gambiae*. The following are several pieces of circumstantial evidence which seem to support this view. In considering some of them the two types of village site described above, near large swamps and near steep banks, should be remembered.

At Sennar, where conditions somewhat resemble those at Kosti, strenuous efforts were being made in 1936 to remove a growth of *E. stagnina* covering several hundred acres and breeding many *A. pharoensis*. It was decided, for the time being, to discontinue this work and to concentrate on the control of *A. gambiae*, although arrangements were made to treat the swamp with Paris green if it proved necessary. Although there is still some malaria this policy of ignoring the swamp is still followed.

Another example which seems to indicate the importance of *A. gambiae* rather than *A. pharoensis* is the state of certain riverain villages in 1941, a year when the rainfall was low and there was thus little inland breeding of *A. gambiae* to interfere with a comparison between villages with different types of river breeding places. The removal inland of four villages near Kawa (Abu Jindi, Muhammad Dioma, Kunuz and Hassan Allob) was being strongly urged owing to severe outbreaks of malaria. All these villages were situated on steep banks near small *A. gambiae*-breeding creeks, and at the same time there was no report of much malaria from Bakht er Ruda, Shabasha and Deneigila, villages situated near large *pharoensis*-breeding swamps.

At Kosti, in October 1942, there was a severe outbreak of malaria apparently due to *A. gambiae* breeding abundantly in pools produced by a sudden drop of 40 cms. in the reservoir level at the end of the rains when gravid females of *A. gambiae* were plentiful. In November the pools dried up and the outbreak declined despite the fact that great numbers of *A. pharoensis* were still sweeping into the town every night from the main swamps.

The educational centre of Bakht er Ruda is situated near the edge of a large swamp, in which great numbers of *A. pharoensis* and, until recently, few other Anophelines bred, not far from an irrigated area in which a few *A. gambiae* are sometimes found. The Province Medical Inspector, in his annual report for 1941, stated that in 1940 and 1941 recorded cases of malaria were only 8 and 6 per cent. respectively and that, although the swamp had bred large numbers of mosquitos, the available evidence indicated that conditions had not deteriorated as a result of the filling of the reservoir but had improved.

From 1943 to 1945 Dr. H. D. Fairman was in charge of medical work in the reservoir area and made some observations on malaria and studied past records. In an unpublished report he stated that in general there was a high splenic index and a low parasite index for villages within one mile of the river or the nearest cultivation. After referring to villages with a high rate of malaria he wrote of a village near Bakht er Ruda. " An exception is Shabasha which, although situated on the river bank within less than ½ mile of a large swamp, shows a low splenic and parasitic index. This is in conformity with the low incidence of malaria recorded at the dispensary. The people complain bitterly of nuisance from mosquitos [almost certainly *A. pharoensis*] and the site appears bad." He stated, however, that the results of the examination of the village should be confirmed.

Referring to the whole reservoir area Fairman wrote " Certainly the increase of malaria does not seem to be commensurate with the vast numbers of the swamp breeding species and there appear to be sufficient *A. gambiae* to account for the increase of malaria." It should also be remembered that any general increase in malaria may have been partly due to *A. rufipes*.

Although there is no precise evidence of the rôle of *A. pharoensis* in the area it seems almost self-evident that this species is much less important than *A. gambiae*. It is a common experience to stay in a town on the Blue Nile, where malaria is prevalent and Anophelines (*A. gambiae*) are very seldom noticed, and to visit a town on the White Nile where there are not many cases in spite of vast numbers of *A. pharoensis*.

A. squamosus.

This species is not an important constituent of the mosquito fauna but it is probably widely distributed. Its identification is not always easy because its larvae closely resemble those of *A. pharoensis*, but adults have been identified from Jebelein, Kosti and Bakht er Ruda. It occurs chiefly in the rainy season and its larvae have been found, usually with *A. gambiae*, in sedge and flooded land grass in the swamps and in grassy rain pools.

Species of Aëdes.

A. unilineatus and *A .vittatus* have been found at Jebelein, *A. aegypti*, *A. metallicus*, *A. arabiensis*, *A. ochraceus* (in an inland swamp) and *A. circumluteolus* at Kosti, and *A. metallicus* and *A. fowleri* at Dueim.

Culex tigripes, C. nebulosus, C. pipiens and subsp. molestus.

C. tigripes has been found breeding during the rainy season in wells, boats and tanks in a few places between Renk and Geteina. The other three mosquitos have been found at Kosti and *C. nebulosus* also at Dueim and Jebel Auliya.

CONTROL MEASURES.

The Jebelein Section.

In this area of extensive swamps and few inhabitants, where the widely-breeding *A. funestus* occurs, intensive house-spraying appears to be the feasible method of control in the few villages that exist.

The Kawa and Geteina Sections.

The removal of villages has already been mentioned.

A. gambiae.

The breeding places of this species are comparatively small and well defined and can be treated with DDT dissolved in oil or with Paris green.

A. rufipes.

Pyrethrum spray is used in houses against this species, which is assumed to be of some importance as a vector of malaria and parts of the swamps are treated with Paris green dust or DDT in oil.

The control of aquatic plants is probably not practicable owing to expenses; also interference with creeping *Vossia* (an excellent fodder grass) which evidently represents the climax of plant succession over great areas, would probably allow *Naias* to grow in large quantities followed by a great increase in mosquitos, particularly *A. rufipes.*

A possible method of partial control is to use cultivators to bank off areas of swamp with permission from the Irrigation Authorities.

A very small quantity of creeping grass and *E. pyramidalis* is removed for fodder.

From observations of the larvae it is evident that treading down of the vegetation should be avoided as much as possible.

Two experiments have been made on the use of *Gambusia affinis holbrooki.* This fish breeds much more readily in captivity than the indigenous *A. laoti* and can therefore be released in large numbers before *A. loati* has had time to colonise the swamps. At Kosti 25,000 *Gambusia* were released at the south end of the sedge swamp north of the town between 5th and 12th September 1945. A month later *Gambusia* could easily be found several hundred metres away and had evidently multiplied greatly. On 16th December large numbers were found one kilometre north of the point of release. The species appeared to have colonised an area of about 20 hectares, but never reached the density attained in stock ponds from which other fishes are excluded. North of this *A. laoti* was abundant and no *Gambusia* was seen. In the areas occupied by both these fishes, mosquito control larvae could easily be found and only partial control resulted from their presence. It seemed probable that *A. loati* had prevented the northward spread of *Gambusia*, and in September 1946 1,000 *Gambusia* were released at Dueim where *A. loati* was thought to be scarce. Three months later *A. loati* was seen to be common and no *Gambusia* were seen.

A. pharoensis.

The treatment against larvae of this species, the control of which is desirable because it probably transmits some malaria and because of the discomfort it causes, is similar to that of *A. rufipes.* Growth of thick vegetation near houses and the stacking of straw near them are discouraged to prevent close association between man and *A. pharoensis.*

Culicines.

The control of potential yellow fever vectors has been discussed by Lewis (1947). *C. poicilipes* is the only troublesome biter and can be partially controlled by the use of DDT in oil.

Larvicidal dusts.

Floatable Paris green has been used for many years in parts of the area. It is applied as a one per cent. mixture in dust, usually by hand but sometimes by hand-

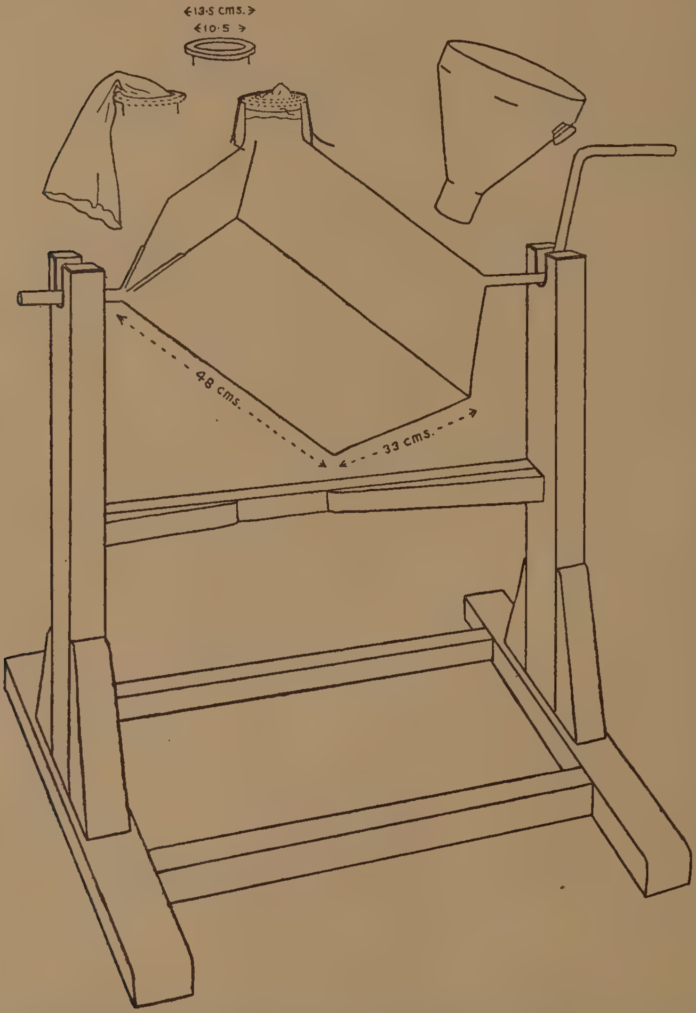

Fig. 3.—Paris green mixer closed by sleeve strapped into opening and showing the handle used as a brake. Small figures show sleeve open, dimensions of opening and funnel for filling.

operated rotary blowers in boats. At present it has certain advantages over some other available insecticidal dusts ; it floats well, can be detected with little difficulty on the water and, even if unduly concentrated, does not kill predators. Other insecticides may soon prove more effective but whatever is used it is considered that for many of the White Nile breeding places dusts are far more useful than liquids.

Dusts can drift with the wind (which usually blows from one direction) over great expanses of *Vossia* grass which is so thick that penetration by boats is difficult. They can be applied to small pools hidden beneath vegetation and to water covered with scum, and in some places to pools where deep soft mud and acacia thorns hinder a close approach. Although the snail hosts of *Schistosoma* are seldom seen in the Kosti and Dueim swamps it is often useful to employ an insecticide which does not necessitate wading in channels which may be infected with cercariae. The infrequency of rain renders dust unlikely to adhere to vegetation instead of falling on the water but, as the White Nile carries very little silt, it is necessary to search carefully for dust for dilution. At Kosti this is obtained from an island.

For Paris green a mixer is used (fig. 3) which was adapted from that figured by Covell (1941). The wide opening and lack of a screw cap facilitates quick filling and emptying and the sleeve used for rapid closing also serves to prevent the formation of a cloud of dust when the mixer is emptied. The mixer is made so that the handle can be slid into the groove of the supporting post to act as a brake during filling. The central horizontal beam is displaced laterally to allow for emptying and the base made to allow room for a tin to receive the mixture.

SUMMARY.

The mosquitos of the reservoir area have been studied mainly with the object of planning suitable methods of controlling malaria. This paper is an account of the work done so far.

The diversity of conditions in the area, which is of such length that it has been divided into three sections, is described with special reference to the river and reservoir levels, predacious fishes, breeding places of mosquitos and water plants among which mosquitos breed.

The mosquito fauna of the area is described and observations on the breeding places and adult habits of the principal species are recorded. The large proportion of " ripe " females among Anophelines dissected is noted.

The probable effects of the reservoir conditions on the mosquitos are discussed.

Notes are given on certain species, and the relation of *A. rufipes* and *A. pharoensis* to malaria are discussed from circumstantial evidence. The former is provisionally regarded as a vector.

Control measures are described with particular reference to *A. funestus* (in the south), *A. gambiae* and *A. rufipes*, the latter being a vector of malaria expensive to control in the larval stage. The value of insecticidal dusts in the area is discussed.

Acknowledgements.

The writer is much indebted to many officials of the Sudan Government who have served in the area ; particularly to Dr. H. Richards and Dr. H. D. Fairman of the Sudan Medical Service for information about malaria and general conditions ; to Miss E. M. Trewvas of the British Museum (Natural History) for the identification of some fishes ; to the Chief of the Research Division of the Department of Agriculture and Forests for many facilities ; and to Dr. F. W. Andrews, Chief Economic Botanist, for the identification of aquatic plants.

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References.

ANDREWS, F. W. (1940). A study of nut grass (*Cyperus rotundus* L.) in the cotton soil of the Gezira. 1. The maintenance of life in the tubers.—Ann. Bot., **4,** pp. 177–193.

——. (1945). Water plants in the Gezira canals. A study of aquatic plants and their control in the canals of the Gezira cotton area (Anglo-Egyptian Sudan). —Ann. appl. Biol., **32,** pp. 1–14.

BAKER, S. W. (1866). The Albert Nyanza Great Basin of the Nile, and explorations of the Nile Sources. I. London.

BALFOUR, A. (1904). First report of the Wellcome Research Laboratories at the Gordon Memorial College Khartoum. Khartoum.

——. (1906). Second report, etc.

BALL, J. (1939). Contributions to the geography of Egypt. Cairo.

BOULENGER, G. A. (1907). The fishes of the Nile. In Anderson, J. Zoology of Egypt, **3–4,** London.

BROUN, A. F. & MASSEY, R. E. (1929). Flora of the Sudan. London.

CASATI, G. (1891). Ten years in Equatoria and the return with Emin Pasha. (Translated.) London.

CHRISTOPHERS, S. R., SINTON, J. A. & COVELL, G. (1941). How to do a malaria survey (4th ed.). Hlth Bull. No. 14 (Malar. Bur. No. 5) Delhi.

Climatological Normals for Egypt and the Sudan. (1938). Cairo.

COVELL, G. (1941). Malaria control by anti-mosquito measures. 2nd ed. Calcutta & London.

EDWARDS, F. W. (1941). Mosquitoes of the Ethiopian Region III.—Culicine adults and pupae. London, British Museum (Natural History).

EVANS, A. M. (1938). Mosquitoes of the Ethiopian Region II.—Anophelini, adults and early stages. London, British Museum (Natural History).

GARNHAM, P. C. C. (1945). The role of *Anopheles pharoensis* Theobald in the transmission of malaria in Kenya Colony.—Ann. trop. Med. Parasit., **39,** pp. 63–65.

HADDOW, A. J. (1942). The mosquito fauna and climate of native huts at Kisumu, Kenya.—Bull. ent. Res., **33,** pp. 91–142.

HENDERSON, L. H. (1932). Some observations on the incidence of malaria amongst the nilotic tribes.—Trans. R. Soc. trop. Med. Hyg., **25,** pp. 281–286.

HOPKINS, G. H. E. (1936). Mosquitoes of the Ethiopian Region I.—Larval bionomics of mosquitoes and taxonomy of culicine larvae. London, British Museum (Natural History).

HURST, H. E. (1944). A short account of the Nile Basin. Cairo.

—— & PHILLIPS, P. (1932). The Nile basin. III. Ten day means and monthly gauge readings of the Nile and its tributaries. Cairo.

LEWIS, D. J. (1939). The male and early stages of *Anopheles wellcomei* Theobald. —Ann. trop. Med. Parasit., **33,** pp. 197–200.

——. (1945). Observations on the distribution and taxonomy of Culicidae (Diptera) in the Sudan.—Trans. R. ent. Soc. Lond., **95,** pp. 1–24.

LEWIS, D. J. (1947). General observations on mosquitos in relation to yellow fever in the Anglo-Egyptian Sudan.—Bull. ent. Res., **37**, pp. 543–566.

LYONS, H. G. (1906). The physiography of the river Nile and its basin. Cairo.

MACDONALD, M. (1920). Nile Control. Cairo.

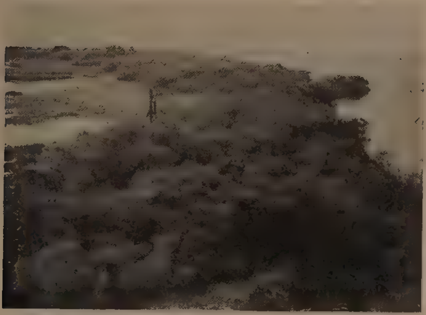
NEWHOUSE, F. (1939). The training of the Upper Nile. London.

PEKKOLA, W. (1919). Notes on the habits, breeding and food of some White Nile fish.—Sudan Notes, **2**, pp. 112–121.

SCHWEINFURTH, G. A. (1874). The heart of Africa. Travels...1868–71. (Translation.) London.

VAGELER, P. & ALTERN, F. (1932). Boden des Nil und Gash V.—Z. PflErnähr. Düng., (A) **23**, p. 149.

WILLCOCKS, W. & CRAIG, J. I. (1913). Egyptian Irrigation I. 3rd edn. London.



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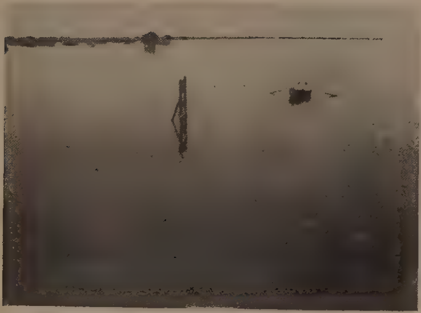

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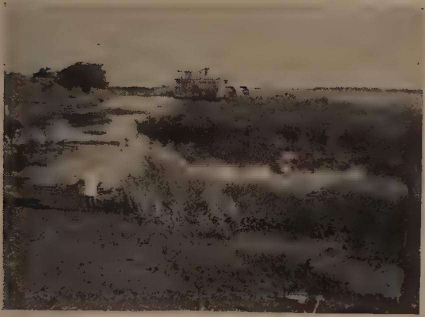

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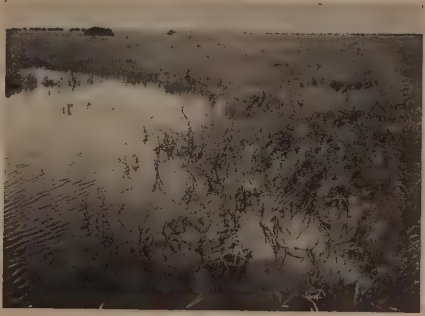

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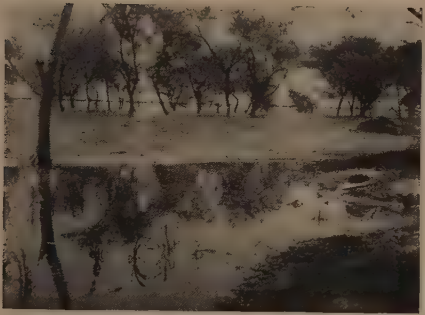

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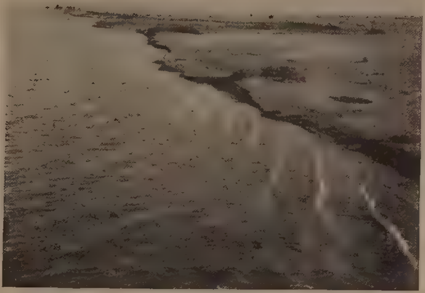


Fig. 6.

Fig. 1. Papyrus and associated swamp seen from Kosti Bridge in December 1937, looking north.
Fig. 2. The same area in October 1945. Papyrus is absent owing to the effect of the reservoir.
Fig. 3. Small swamp protected by papyrus in the Gulli area in December 1938.
Fig. 4. *Vossia* swamp at Kosti in 1945.
Fig. 5. Pools formed by wave action at Dueim in December 1938.
Fig. 6. Pools formed by wave action near Geteina in October 1945.

STUDIES ON THE BEHAVIOUR OF ADULT AUSTRALASIAN ANOPHELINES.

By F. H. S. ROBERTS and P. J. O'SULLIVAN,
Animal Health Station, Yeerongpilly.

The outbreak of war in the south-west Pacific area found the Allied Forces with but a very meagre knowledge of the relationship of Anophelines to malaria in the Australasian zone. Heydon (1923) and de Rook (1926) (quoted by Swellengrebel and Rodenwaldt, 1932) had implicated *Anopheles punctulatus farauti*, Laveran, and *A. punctulatus punctulatus*, Dönitz, as the principal vectors in New Britain and Dutch New Guinea. In 1942, during a benign-tertian epidemic in the Cairns area, North Queensland, Heydon (unpublished) found a sporozoite rate of 0·66 per cent. among 1,535 *A. punctulatus farauti.* At this time, also, a single female of 79 *A. amictus hilli*, Lee and Woodhill, was found with sporozoites.

This information enabled Army Malaria Control Units to deal effectively with the breeding places of the vector species in areas where they occurred. It was realised, however, that, for control to be brought to the highest point of efficiency, consideration must also be given to the adult Anophelines. Here the only relevant information was confined to a few observations by Heydon (1923) at Rabaul, New Britain, where it is recorded that adults of both varieties of *A. punctulatus* frequent houses throughout the night to feed and, during the day, are most easily found resting in dark situations in huts and rooms used by natives.

Atherton and Lemerle (unpublished Army Reports, 1943) provided more detailed information on adult behaviour as a result of their observations on *A. punctulatus farauti* at Lalapipi, New Guinea. They showed that during the day, this mosquito could be found in large numbers resting in sheltered spots in the bush, close to habitations. It did not appear to remain in tents and huts any longer than was required to feed. At about the time these studies were being made by Atherton and Lemerle in New Guinea, the authors, who were then members of the 2nd Australian Mobile Entomological Section, A.A.M.C., were working along similar lines at Cairns, North Queensland. Later, an opportunity arose for further observations at Salamaua and Mirivasi in New Guinea.

OBSERVATIONS AT CAIRNS, NORTH QUEENSLAND.

The city of Cairns is built only a few feet above sea level, and much of it is intersected with low-lying, swampy areas. At the time of these investigations, the majority of these swamps were covered with vegetation, mainly reeds and hyacinth, and were favoured breeding places of *A. bancrofti bancrofti*, Giles. This species, in the Cairns area, rarely attacks man, though on one occasion a fair series was caught by using a horse as bait. For this reason it was not considered to be an important vector of malaria.

In the south-western section of the city, the Bungalow suburb, many of the swamps were reached by high tides, and the resulting brackish waters were a prolific source of *A. punctulatus farauti.* During the early part of the year, large numbers of *A. amictus hilli* also bred there.

Our observations were confined to these last two Anophelines, and were made from January to April, 1944, when both were very prevalent. A catching station was established in the Bungalow suburb, close to a street of occupied houses, approximately 400 yards from a large brackish swamp in which both were breeding plentifully.

Adult Activity at Night.

Catching station records.

The method adopted in catching adults in the open at night was to sit and wait until they had alighted and were attempting to bite. They were captured in small glass tubes, and transferred to cages.

Both Anophelines show a decided preference for the lower portions of the body, and the majority of our specimens were taken after alighting on the boots, gaiters, or trousers below the knees. Some idea of their prevalence is given in Table I, in which is recorded a series of catches, and the respective biting rates, between 7.30 p.m. and 9.30 p.m. (E.S.T.).

TABLE I.

Prevalence of adult Anophelines at catching station 7.30 p.m. to 9.30 p.m. (E.S.T.)

Date	*A. amictus hilli*		*A. punctulatus farauti*	
	No. taken	Biting rate*	No. taken	Biting rate*
3. ii.44	101	13	42	5
5. ii.44	163	20	139	17
15. ii.44	127	16	163	20
24. ii.44	84	10	360	26
6.iii.44	93	12	311	76
10.iii.44	19	5	300	75
13.iii.44	4	0·25	322	33
22.iii.44	0	0	81	20
28.iii.44	0	0	161	40
4.iv.44	0	0	132	44
11.iv.44	0	0	72	36

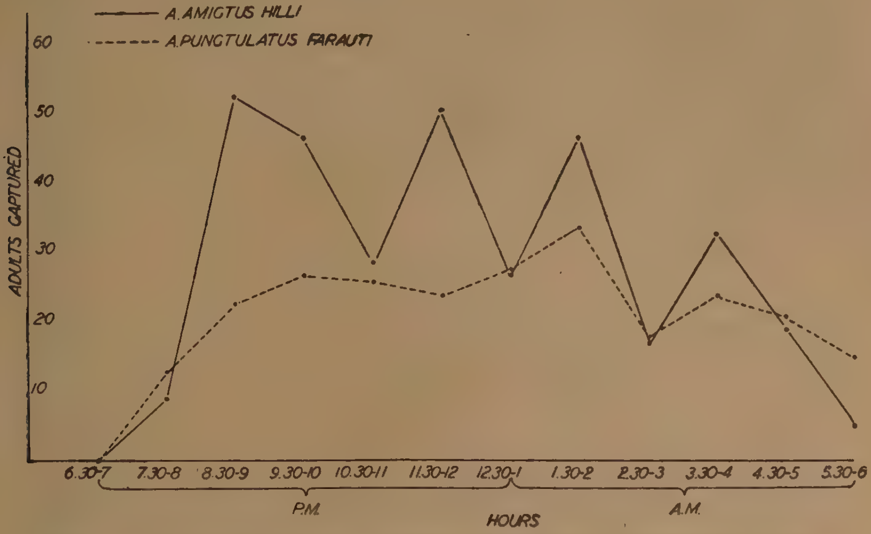
*Biting rate=biting rate per man per hour.

When these catches commenced, *A. amictus hilli* was very prevalent, but decreased considerably between the 6th and 13th March and thereafter apparently disappeared. *A. punctulatus farauti* reached a biting rate of 76 per man per hour on the 6th March and thereafter also decreased in numbers, but nevertheless remained fairly prevalent. This decrease was possibly due to the institution of control measures, for later on, as control became more intensified, this mosquito practically disappeared. The decrease of the *A. amictus hilli* populations was brought about by heavy rains which flooded the breeding area, rendering it unsuitable for this Anopheline.

Prevalence throughout the night.

It was generally believed that Australasian Anophelines were most prevalent just at dusk and again at dawn. To ascertain whether any such periodicity did occur, an all-night catch was carried out from 6 p.m. to 6 a.m. (E.S.T.).

Catching was limited to a half-hour period in each hour, *e.g.*, 7.30 p.m. to 8 p.m., 8.30 p.m. to 9 p.m., etc., and the Anophelines were captured in the manner already mentioned. The results are expressed in Graph 1.



Graph 1.—Nocturnal activity of *A. punctulatus farauti* and *A. amictus hilli* at Cairns.

The first females of both Anophelines did not arrive until about 7.15 p.m., just when it was getting dark. In the case of *A. punctulatus farauti*, the numbers were fairly even from 8.30 p.m. to 5 a.m., with a small peak of abundance at 1.30 to 2.0 a.m. The small number captured between 5.30 and 6 a.m. indicates that activity decreased during the period immediately prior to daylight.

The catches of *A. amictus hilli* show that, on the night the catch was made, the adults arrived in waves, being most prevalent between 8.30 and 10 p.m., 11.30 and midnight, 1.30 to 2 a.m. and again, though to a lesser extent, between 3.30 to 4 a.m. Just prior to daylight the numbers fell considerably.

Effect of rain on prevalence.

Both mosquitos continue to be active during light showers. Activity ceases during heavy rain, but may be resumed immediately the rain stops. Heavy continuous rain has little effect on abundance. For example, on 8th February, a three-hour catching period from 7.30 to 10.30 p.m. yielded 109 adults of *A. amictus hilli* and 126 adults of *A. punctulatus farauti*. Fourteen inches of rain were recorded over the next five days, and on the night of 13th February, a few hours after the rain had ceased, 126 *A. amictus hilli* and 162 *A. punctulatus farauti* were taken during the same three-hour period by the same catching team.

Biting habits.

A. punctulatus farauti is very wary in its attack, and very readily disturbed. *A. amictus hilli*, on the other hand, is more direct in seeking a blood-meal, and more easily captured.

During periods of activity, adults of both may be seen resting on grass and weeds near the catching party. Two females of *A. amictus hilli* were timed to rest at least 42 minutes, but usually the period of rest is shorter than this.

Behaviour indoors.

Both mosquitos will enter houses in search of a blood meal. They will fly into brightly lighted rooms, but prefer the more shaded places as, for example, beneath a table. They may be seen resting on walls, articles of furniture, curtains, etc.

There are indications, however, that the number entering brightly lighted rooms of houses is only a small proportion of the existing populations. This is shown in Table II, which compares the numbers caught by two men in the lighted room of a house and by two others at ground level immediately outside the house between 7.30 and 9.30 p.m. (E.S.T.). The four men changed positions for the second catch.

TABLE II.

Relative abundance of Anophelines in the open and in a house.

Date	*A. amictus hilli*		*A. punctulatus farauti*	
	No. in house	No. in open	No. in house	No. in open
6.iii.44	8	23	25	305
13.iii.44	1	6	13	123

The differences are all the more significant because the five occupants of the house were also present in the room in which the catch was made. The house in which these figures were obtained was about 7 feet above ground level. The comparatively small numbers obtained there may be simply an indication that these Anophelines usually fly close to the ground, rather than of any reluctance to enter a house or a brightly lighted room.

Diurnal Resting Places.

The observations recorded here were made between 7.30 a.m. and 10 a.m. (E.S.T.).

Sheltering in houses.

Considering the large populations of Anophelines prevalent during the time these observations were made, only a few were found sheltering in houses.

In the case of *A. amictus hilli*, only one female was taken in an occupied house. Seven others were captured in huts occupied by service men within the breeding areas; these females were sheltering in dark corners, usually close to the floor, behind furniture, bags of clothing, etc. Seven had fed the previous night, and one contained the remnants of a blood meal.

Only three females of *A. punctulatus farauti* were captured in houses during the day. These were all engorged, and were resting on the wall behind furniture and clothing. Eight further females, four of which had recently fed, were located close to the floor of a detached bathroom, where a leaking shower was providing apparently attractive conditions of humidity.

Sheltering in the bush.

The first female *A. punctulatus farauti* discovered in this investigation was sheltering in a stack of timber underneath a house. Numerous males also occurred here. The favourite resting habitat, however, proved to be in the trash of banana plants growing close to the houses. Here both males and females were found resting in small depressions in the earth, under the corms of the trees, and among the trash, close to the ground where it was sheltered and moist. A few specimens were found between decumbent trunks and leaves up to 2 feet above the ground. Males outnumbered females. Careful searching in the surrounding dry bush failed to reveal a single adult. Males have been found resting up to 600 yards from the nearest breeding area.

Of 211 females examined, 77 had fed the night prior to capture, and many of the others contained traces of old blood-meals. Good catches were usually made after overnight rain. On dull mornings, some of the females, when disturbed, attempted to bite.

In contrast to the large number of *A. punctulatus farauti* found sheltering in the above type of habitat, only 11 female *A. amictus hilli* were taken. Of these, seven had fed the previous night and three contained traces of blood-meals.

Sheltering in the vicinity of the breeding area.

These observations were confined to a large swamp of brackish water (840 parts chloride per 100,000) in which both species were breeding in very large numbers. The swamp covered an area of approximately ten acres. It was comparatively shallow throughout, and a large portion was open to sunlight, though littered with dead mangrove stumps and clumps of dead reeds.

A preliminary search among the thick grass and litter extending back from the edge of the swamp brought to light several male and female *A. amictus hilli* and a solitary unfed female *A. punctulatus farauti*. This was the only specimen of the latter found here. On the other hand, the swamp proved a very prolific source of *A. amictus hilli*. Numbers of males and females were found not only in the grass around the swamp, but also resting on the mangrove stumps and reeds in the water itself, usually on the shady side only a few inches above the surface of the water. The clumps of reeds were greatly favoured, and on one occasion 34 females were taken by two men from a small clump in 30 minutes. During showery weather, and even after heavy overnight rain, adults could still be seen resting in the swamp.

As a rule, these adults were easily captured, but once disturbed became very wary. Their flight is low, fairly direct, usually only for a short distance, and readily followed with the naked eye. On dull mornings they may attempt to bite.

Of 101 females examined, only one showed evidence of a recent blood-meal, whilst the majority had traces of old blood-meals.

Determination of Age of Female Anophelines.

Throughout, we endeavoured to secure some idea of the ages of the females captured, and to correlate the estimated ages with the habitats in which the mosquitos were found ; for it was realised that, if this could be accomplished with reasonable accuracy, valuable information on adult bionomics would be obtained.

Criteria employed for age determination.

For this purpose, we endeavoured to employ four criteria, namely, condition of the wings, development of the ovary, presence or absence of blood in the midgut, and the presence or absence of spermatozoa (Christophers, Sinton & Covell, 1944).

Condition of the wings.—After a careful examination of some thousands of wild Anophelines in Australia and New Guinea, we have come to the conclusion that any attempt to determine age on wing condition alone would be most unreliable. We have seen well-developed oocysts in females with Stage I wings, and a gland infection in a female whose wings were barely Stage II. These females would be at least 8 to 12 days old. We have also observed Stage I wings on mosquitos which have laid at least one batch of eggs, and are therefore at least 5 days old (see below), and an occasional female, which has not yet laid, with wings as worn as Stage III. By far the majority of the females seen by us had either Stage I or Stage II wings, only a few had Stage III wings, whilst Stage IV was rare. We have yet to encounter a specimen with wings in Stage V. In all probability, the proportion of Stage I wings among our mosquitos was greater than recorded, for the mosquitos were captured in glass tubes, and then placed in cages where they sometimes remained for several hours before examination.

Ovary development.—As a basis for interpretations of ovary development in respect to age, a series of observations were carried out in the laboratory with bred *A. punctulatus punctulatus*. These studies showed :—

1. That Stage II is reached within 24 hours of emergence.
2. That development and maturation of the ovary will proceed in the absence of fertilisation. Only one batch of eggs matures, however. These may be retained by the female for the duration of her life in captivity.
3. Maturation of the ovary occurs only after a blood-meal. Otherwise, development does not proceed beyond an early Stage II. Multiple blood-meals, at least two, are required by many mosquitos before the majority of the follicles mature.
4. After a blood-meal and under summer conditions, complete maturation of the follicles occurs on the third or fourth days, there being an interval of about one day between each of the stages.

Digestion of blood-meal.—Few females seek a blood-meal during the first 24 hours of life. Some feed when 24 to 36 hours old, but the majority not until about 48 hours old. Females may feed every two days.

Under summer conditions, complete digestion of a blood-meal is accomplished in 3 to 4 days.

Presence of spermatozoa.—An examination of the spermotheca is of value in two ways. On the one hand, absence of spermatozoa confirms other evidence of recent emergence. On the other hand, empty spermathecae have been found in females with what we have designated as Stages II+V or III+V ovaries, the retained, mature eggs indicating recent oviposition. It is most improbable that these females would have exhausted all the spermatozoa on one batch of eggs, and we therefore considered that they must have been at least 8, and possibly 11 or more, days old. It seems clear that a single fertilisation is not always sufficient to last throughout life.

From these observations we have arrived at the following conclusions :—

1. Recently emerged mosquitos have Stage I wings and Stage I (or early Stage II) ovaries. There will be no blood in the midgut if the female is under 24 to 36 hours old. The absence of spermatozoa in any specimen with Stage I or early Stage II ovaries indicates that emergence is very recent.
2. Mosquitos with Stage V ovaries are at least 4 to 5 days old.
3. Mosquitos with Stage II and sometimes Stage III ovaries, and also one or more retained mature eggs indicating recent oviposition, are at least 5 to 6 days old.
4. Females with Stage III ovaries and without any evidence of blood in the midgut have laid at least one batch of eggs and are then at least 6 days old.
5. Females with Stage II ovaries and one or more retained mature eggs, and without spermatozoa, have probably laid at least two batches of eggs and are at least 8 days old.

Whilst studying the problem of age determination with captured wild *A. punctulatus farauti* in New Guinea, it occurred to us that the Stage II ovaries could be divided into three distinct groups, each of which may have some bearing on oviposition and therefore on age. These groups are defined below :—

1. *Stage IIA Ovaries.*—Here the oviducal canal is invisible, the follicles are tightly packed, and the yolk granules are very few and scattered. A female with this class of follicle is considered to be very young, and not yet to have taken a blood meal.

2. *Stage IIB Ovaries.*—In this class the oviducal canal is distinct, and usually well developed, indicating that mature eggs have recently passed into the ovarian tube. The yolk granules vary from few and scattered to numerous and relatively dense. The nucleus, however, is always plainly visible. A female with such an ovary is considered to have laid very recently. The evidence for this view was obtained from the following observations :—

    (*a*) Wild engorged females, set aside for oviposition, show, after the eggs have been laid, a very conspicuous oviducal canal and ovaries in early Stage II. In such females, the gut may be entirely empty, or contain only a trace of old blood.

    (*b*) In captured wild females with Stage II ovaries and with one or more mature eggs, the oviducal canal is usually well developed.

3. *Stage IIC Ovaries.*—In ovaries of this type, the yolk granules are dense, and almost obscure the nucleus. The oviducal canal cannot be seen. If the gut is empty, or contains blood remains, this class of Stage II ovary is considered to belong to a female which has laid at least one batch of eggs, the interval between oviposition and examination being sufficiently long for the oviducal canal to have contracted and become indistinguishable. On the other hand, if there is evidence of a recent blood-meal, a class IIC ovary with the oviducal canal invisible may indicate development of the first batch of eggs.

These interpretations are recorded merely as suggestions, which may be considered worthy of further attention. We have not been able to follow them any further than as set out above. They were made after the work at Cairns had terminated, and we have not been able to apply them to the studies made in that area.

Application of criteria of age to captured females.

Examinations of representative samples of the populations from the habitats mentioned above are set out below.

Wing Condition.—As mentioned previously, the Anophelines captured at night were caged and not examined till next day. Many of the Stage II wings recorded may, therefore, have been in Stage I when the insects were caught. Those taken in diurnal resting places were examined within an hour or two of capture.

In the case of *A. amictus hilli*, approximately 97 per cent. of the females captured at night had either Stage I or Stage II wings, the remaining 3 per cent. being in Stage III. Of those from diurnal resting places, 71 per cent. had Stage I wings, 27 per cent. Stage II, and 2 per cent. Stage III.

With *A. punctulatus farauti*, 98 per cent. of the night catches had Stage I or Stage II wings and 2 per cent. Stage III, whilst the day catches yielded less than 1 per cent. Stage III, the remainder having Stage I or Stage II wings, the former predominating.

Ovary Development.—The results of dissections for ovary development are given in Table III.

TABLE III.

Ovary Development.

Species	Habitat		No. examined	Ovary development—per cent.				
				Stage I	Stage II	Stage III	Stage IV	Stage V
A. amictus hilli ...	Night catches		271	13	63	9	6	9
	Day catches	houses ...	7	—	25	45	15	15
		bush ...	11	9	—	45	18	18
		breeding area	101	2	6	36	6	50
A. punctulatus farauti	Night catches		241	10	82	5	1	2
	Day catches	houses ...	11	—	60	12	14	14
		bush ...	152	20	32	28	8	12
		breeding area	1	—	100	—	—	—

No traces of blood were present in any of the females with Stage I ovaries, and these may be safely considered as young mosquitos of from 24 to 36 hours old seeking their first blood-meal. Thirteen per cent. of the *A. amictus hilli* and 10 per cent. of *A. punctulatus farauti* taken at night fall into this category. The highest proportion of recent emergences occurred in the bush daytime resting habitat of *A. punctulatus farauti*, 20 per cent. of the females taken here having Stage I ovaries and no trace of a blood-meal. In the cases of *A. amictus hilli*, on the other hand, only 1 of the 11 females resting in the bush was a recently emerged mosquito. The majority of the females of both Anophelines taken at night had Stage II ovaries; a high proportion was in very early Stage II, without any signs of blood in the mid-gut, and these are therefore considered as recent emergences seeking their first blood-meal. In a few, the presence of a few mature eggs showed that oviposition had occurred recently and that these females were at least 5 to 6 days old.

The proportion of females resting in the bush and houses during the day with Stage IV and Stage V ovaries was much higher than among the females taken at night. This might be expected, as many of the former had fed and were awaiting maturation of the ovaries. Some of both Anophelines resting in the bush had Stage II ovaries with one or more mature eggs, and had no doubt recently oviposited and returned to shelter.

The figures obtained from *A. amictus hilli* captured in the breeding area show that only 2 per cent. were recent emergences. The population was composed largely of females with Stage V ovaries and therefore about to oviposit (52 per cent.). The remaining 48 per cent., with Stage II, III and IV ovaries and containing traces of blood, had no doubt already oviposited. The ages of the population here would vary from about 24 hours to at least 6 days.

Fertilisation.

The analysis of the figures obtained from dissections for the presence or absence of spermatozoa is shown in Table IV in relation to ovary development.

In the case of *A. amictus hilli*, 12 per cent. of the females with Stage I and 19 per cent. with Stage II follicles had not been fertilised. These females had just recently emerged, and had not yet encountered any males. Some of these were captured in the open at night and others by day resting in the bush. Of eight females with Stage II+V follicles, indicative of recent oviposition, two showed no spermatozoa.

TABLE IV.

Fertilisation in relation to ovary development.

Species	No. examined	Per cent. showing spermatozoa				
		Stage I ovary	Stage II ovary	Stage III ovary	Stage IV ovary	Stage V ovary
A. amictus hilli	111	88	81	100	100	100
A. punctulatus farauti ...	286	42	94	94	100	90

With *A. punctulatus farauti*, a high proportion, namely 58 per cent., of the females with Stage I follicles was unfertilised. This was especially pronounced among those resting during the day in the bush, where no less than 73 per cent. of those with Stage I follicles showed no spermatozoa. It was surprising to find that 10 per cent. of the females with Stage V ovaries were unfertilised, which may indicate that a proportion of females may not encounter males for some days after emergence (see, however, p. 164). Six females with Stages II+V follicles all yielded large numbers of spermatozoa.

Host Preferences.

The presence of newly fed females in the diurnal habitats gave an opportunity for obtaining some information on the source of the blood-meals. The ingested blood was absorbed on filter paper and forwarded to Dr. G. A. M. Heydon, School of Public Health and Tropical Medicine, University of Sydney, for the precipitin test.

Most of the samples taken came from the bush habitat. Some were from females resting in houses. In addition to man, horses, goats, cows, dogs, toads, fowls and ducks were available as a source of blood. Dr. Heydon's report is summarised in Table V.

TABLE V.

Source of blood-meal.

Species	No. Samples	Man	Horse	Ruminant	Dog	Fowl or Duck
A. amictus hilli ...	8	8 (100%)	0	0	0	0
A. punctulatus farauti	81	7 (9%)	18 (22%)	21 (26%)	5 (6%)	30 (37%)

Little weight can be attached to the results given for *A. amictus hilli*, as all of the 8 samples came from females taken resting inside occupied houses and army huts.

In the case of *A. punctulatus farauti*, the largest numbers fed on ruminant and fowl or duck. These hosts were the nearest to the habitats in which the engorged females occurred. From these results, it seems that *A. punctulatus farauti* may prove to have very little host preference, and will feed on the nearest available host. This opinion is supported by a series of 88 samples taken by Atherton and Lemerle from females resting in the bush at Lalapipi. Of these, 19 per cent. were positive for man, 47 per cent. for dog, 33 per cent. for pig and 1 per cent. for cat (unpublished Army report).

OBSERVATIONS AT SALAMAUA, NEW GUINEA.

During service in New Guinea, the authors carried out a considerable amount of work on the use of DDT for controlling adult Anophelines. These investigations involved a careful study of the behaviour of adults in relation, more particularly, to their feeding habits both indoors and outdoors.

Observations were made at Salamaua during May to July and at Mirivasi from August to October, 1944.

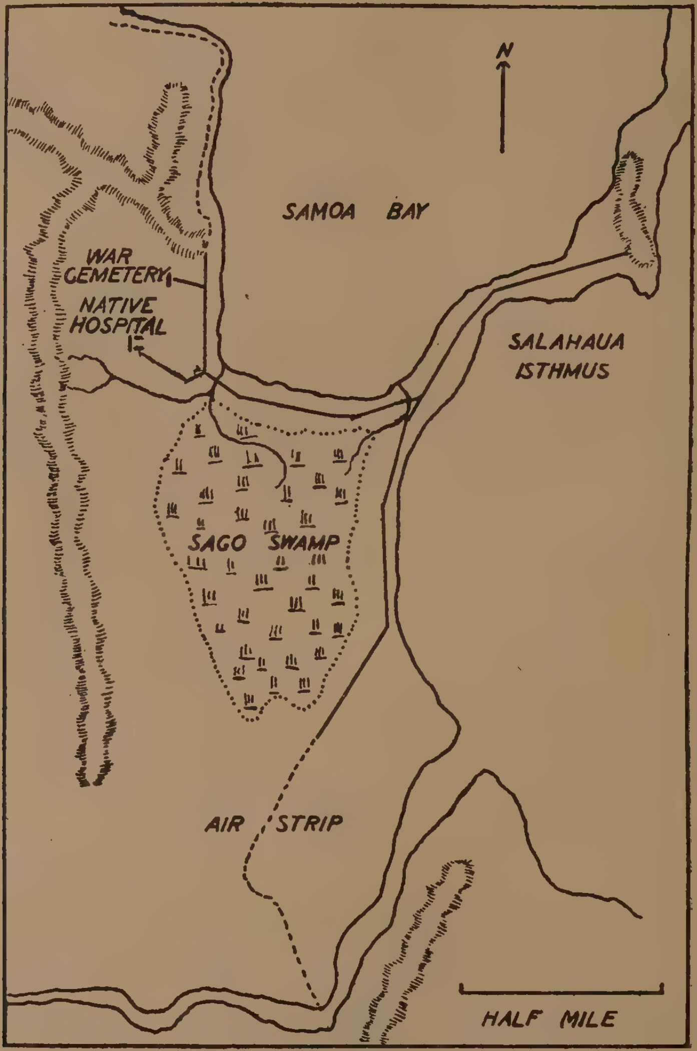

N
SAMOA BAY
WAR CEMETERY
NATIVE HOSPITAL
SALAHAUA ISTHMUS
SAGO SWAMP
AIR STRIP
HALF MILE

Fig. 1.—Map of Salamaua area, showing localities mentioned in the text.

Salamaua is situated on Samoa Bay on the north coast of New Guinea. On the east, Samoa Bay is bounded by a narrow isthmus about 1¼ miles long. At the time of the investigations, about 50 Army personnel and a number of natives were housed on the isthmus. On the opposite side of the bay, and close to its shores, were a native hospital and a war cemetery. The native hospital consisted of a hospital ward housing up to 50 patients, a hut in which the native attendants were quartered, and a hut for the white hospital orderly. The war cemetery was a little more than one-quarter of a mile north of the hospital and was attended by a Graves Registration Unit of 7 men. All huts were of the usual native construction. Towards the south, and about one and a half miles from the base of the isthmus, was an emergency landing strip. These features are shown on the map of the area in fig. 1.

Anophelines Present.

A. punctulatus punctulatus was breeding freely in pure culture in the strip area, and was also the only Anopheline bred from the area to the north of the cemetery. Both here and on the strip, breeding was confined to a series of pools in wheel tracks. In the hospital and cemetery areas also, larvae of this species were very numerous. A few larvae of *A. punctulatus farauti* were taken from the edge of an extensive sago-palm swamp between the hospital and the landing strip and from pools in the vicinity of the hospital.

Adults captured in the hospital and cemetery included *A. punctulatus punctulatus*, *A. punctulatus farauti* and intermediate forms. Of approximately 1,000 adults, 54 per cent. were *A. punctulatus farauti*, 27 per cent. *A. punctulatus punctulatus* and 19 per cent. intermediates (Woodhill, 1946).

Adult Behaviour at Night.

Catching-station records.

Catching stations were established in the open close to heavy breeding grounds in the vicinity of the air strip, the hospital and the cemetery. The methods that proved so successful at Cairns were used. Three to four men comprised the catching team, and the period of catching extended to 1 a.m. The results were very disappointing. The largest number of adults captured at any period was 7 *A. punctulatus punctulatus*, which were taken on the air strip between 11.45 p.m. and 12.15 a.m. during light rain.

It was not until catching stations were established within the hospital and cemetery huts that Anophelines were seen in any numbers. Here, both subspecies and intermediates were taken. On clear, calm nights the catches were high, but they fell off considerably with any breeze and subsequent lowering of the temperature, or during heavy rain. Some idea of adult prevalence is given in Table VI.

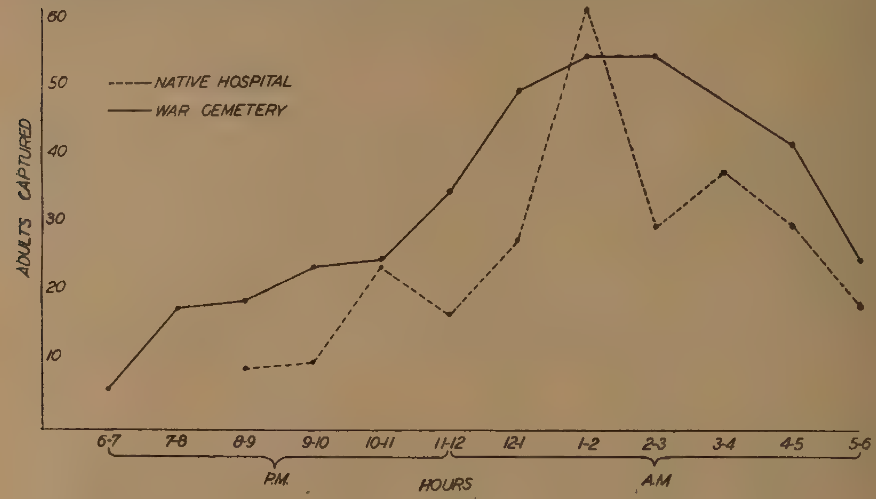
TABLE VI.

Adult prevalence at hospital and cemetery—10 p.m. to midnight.

Date	Catching Station	No. adults captured	Biting Rate
12. vi.44	Hospital	54	7
28. vi.44	,,	60	10
1.vii.44	Cemetery	30	5
7.vii.44	,,	54	9
9.vii.44	,,	18	3
11.vii.44	,,	42	7
12.vii.44	,,	102	17
13.vii.44	,,	60	10
24.vii.44	Hospital	240	40
20.vii.44	,,	306	51
26.vii.44	,,	372	62
28.vii.44	,,	144	24

Nocturnal Prevalence.

Two all-night catches were attempted to study nocturnal prevalence, one at the hospital, the other at the cemetery. Catching was carried out for the full period of 8 p.m. to 6 a.m. at the hospital and 6 p.m. to 6 a.m. at the cemetery. The results are shown in Table VII and Graph 2. As there were no significant differences in the periodicity of the two subspecies and intermediate forms, the points plotted in the graph represent the total number of Anophelines captured for each hourly period.



Graph 2.—Nocturnal activity of *A. punctulatus* (races combined) at Salamaua.

TABLE VII.

Nocturnal prevalence—Four men catching. Hospital, 12th June, 1944.

Species	6–7 p.m.	7–8 p.m.	8–9 p.m.	9–10 p.m.	10–11 p.m.	11–12 p.m.	12–1 a.m.	1–2 a.m.	2–3 a.m.	3–4 a.m.	4–5 a.m.	5–6 a.m.
A. punctulatus farauti	—	—	8	8	21	11	19	39	23	23	19	6
A. punctulatus punctulatus	—	—	0	1	2	4	3	15	5	10	7	10
A. punctulatus intermediates ...	—	—	1	1	1	2	6	8	2	5	4	2
Totals	—	—	9	10	24	17	28	62	30	38	30	18
Cemetery, 28th June, 1944.												
A. punctulatus farauti	4	9	9	7	11	12	13	25	30	24	19	12
A. punctulatus punctulatus	2	7	8	12	8	16	25	17	17	15	12	4
A. punctulatus intermediates ...	0	2	2	5	6	7	12	13	8	9	11	8
Totals	6	18	19	24	25	35	50	55	55	48	42	24

Both catches, including both subspecies and intermediates, showed a gradual increase during the early hours of the night, to become most prevalent between midnight and 3 a.m. In both localities, there was a sharp decrease between 5 and 6 a.m.

There is some similarity between the picture of prevalence presented here and that obtained at Cairns. In both cases, the peak of abundance occurred in the early hours of the morning, though at Cairns this was not as marked as at Salamaua. In both catches at Salamaua the adults arrived in waves. During any hour, there was always a period of 10 to 15 minutes when mosquitos were most numerous.

Feeding Habits.

At Cairns it was observed that the number of *A. punctulatus farauti* entering a brightly lighted room in a house was only a small proportion of the existing population. At Salamaua, on the other hand, observations showed that the only profitable catching stations were inside huts. These huts were built close to the ground, and, with their open doorways and windows, and walls ending 2 to 3 feet from the roof, provided easy access to hunting mosquitos. They were lit at night by hurricane or native oil lamps, and the light was dim.

At Cairns, it was also noted that *A. punctulatus farauti* may rest after arriving to feed and possibly also between bursts of activity in attempting to feed. At Salamaua, of 728 adults captured in huts at night, 431 (*i.e.* 59 per cent.) were taken resting unfed. The positions selected for resting included the walls and roof and any article of furniture present. The roof appeared to be the least favoured, but adults have been seen up to a height of about 9 feet. After feeding, females rest again close to their host, and, when disturbed, fly only a short distance. Both engorged and unfed adults have been timed to rest as long as 72 minutes. The majority of unfed mosquitos, however, rested from 5 to 20 minutes.

The time taken for complete engorgement ranged from 2 to 4 minutes, with an average of about 3 minutes.

Host Preference.

During catching at the hospital, there was evidence that these Anophelines are more readily attracted to natives than to white men. This is instanced by the following observations :—

1. On several occasions, catches by four white men in a hut 20 yards from the native hospital yielded only a few adults, even though catching periods were extended into the early hours of the morning. A native catching in the hospital for only 20 to 30 minutes during these attempts would take 30 to 40 females.
2. At one end, the hospital hut was partitioned off by a low wall to form a dispensary. On two occasions, two white men captured 3 and 5 adults respectively here in an hour, then walked a few yards into the ward and took 30 to 60 adults resting on the walls in 45 minutes.
3. In a tent set up a few yards from the hospital, 2 white men captured 2 adults in an hour. In the hospital itself adults could be seen in large numbers.

It is not apparent whether these observations are the result of preference, or simply an expression of the amount of unwashed, sweaty skin exposed ; but it was a frequent experience in New Guinea that many more adult Anophelines could be caught in native quarters than among even high concentrations of white men.

Flight Range.

During the 1942 malaria epidemic at Cairns, an attempt was made to determine the flight range of *A. punctulatus farauti*. Adults were found half a mile from the breeding grounds, but catching stations one mile away yielded negative results.

At Salamaua, isolated females of *A. punctulatus farauti* were not uncommon in the huts on the isthmus. The nature of the ground was such that we could be reasonably certain that there were no breeding grounds on the isthmus itself, the nearest being the large sago-palm swamp about one mile away.

Diurnal Resting Places.

The poor results obtained at night from catching stations established in the immediate vicinity of breeding grounds, and the apparent concentration of adults at the hospital and cemetery, is interpreted as evidence that adults leave the breeding areas shortly after emergence in search of a blood-meal, and tend to congregate in the vicinity of human settlements, providing, of course, that suitable daytime resting places are available. They leave here only for oviposition and to feed. At Cairns, where large numbers of *A. punctulatus farauti* were captured in the open, it would now seem that this occurred only because the breeding grounds, source of blood-meals, and diurnal resting places all occurred in the same area.

As evidence that adults were resting in numbers in the bush in the immediate neighbourhood of the hospital, (*a*) no adults could be found in the huts during the day, and (*b*) a control experiment, in which the vegetation for 200 yards around the hospital was sprayed with DDT, brought about a 60 per cent. reduction in the population visiting the hospital the night subsequent to spraying. The population was normal again the next night, indicating that new mosquitos had moved in from the breeding areas. Other observations at Salamaua and Aitape (Ratcliffe, Gilmour & Mules, unpublished) suggest that the rate of infiltration by *A. punctulatus* is about 200 yards per day.

The daytime resting places are in all probability similar to those observed at Cairns, for a few females were found resting in well sheltered, moist spots amongst the vegetation close to the ground on the banks of a creek near the hospital.

That both the subspecies and intermediate forms may, however, occur at times in large numbers in huts during the day, is instanced by an examination of a native compound at Nadzab, near Lae. This compound consisted of a number of huts with earthern floors, surrounded by dense jungle, and with a sluggish creek nearby. The huts were open at both ends and for about 6 feet on each side. Large numbers of engorged females, chiefly *A. punctulatus farauti*, were seen resting in moist holes in the ground, inside boxes and on the walls and roof.

OBSERVATIONS AT MIRIVASI, NEW GUINEA.

Mirivasi is on the southern coast of New Guinea at the mouth of the Lakekamu River, in the Gulf of Papua. It is close to Lalapipi, where Atherton and Lemerle made their earlier observations. Except for a narrow coastal strip of dry land, the area is one huge sago-palm swamp, which extends many miles inland. The period of our stay in this part of New Guinea occurred during the season of the south-east trades. Strong winds frequently prevailed for the whole day, but usually ceased during the early hours of the morning until just after sunrise.

Adult Habits.

A. punctulatus farauti was extremely prevalent here. The only other species of Anopheline seen was *A. subpictus*, Grassi, which was present in moderate numbers.

Anophelines were not seen in any numbers until the strong winds subsided in the early hours of the morning. On several occasions unfed adults were observed resting in huts as at Salamaua and Cairns.

A search for diurnal resting places revealed many adults in the immediate vicinity of two native villages. In one case, they were resting close to the ground beneath an accumulation of fallen palm fronds on sandy soil. In the second village, a number was found under vegetation and débris on almost wet soil in a well shaded area.

A search of the more open huts during the day revealed only one female. Engorged females were not uncommon, however, in small, dark huts, in which the doors were shut and windows non-existent. It is presumed that huts of this type acted as traps, the mosquitos finding their way in through various cracks and crevices, but being unable to make their way out after feeding.

Age of the Anopheline Populations.

The purpose of the studies at Mirivasi was to determine the effect on the malaria rate in a native village by the treatment of hut walls with DDT. Anopheline infection rates and spleen and parasite rates were compared in two native villages, one of which was subsequently treated with DDT. The work was carried out in co-operation with the 3rd Medical Laboratory, U.S. Forces.

In view of the extremely large populations of *A. punctulatus farauti* that were present in this area and the moderately high gametocyte rate which existed among the natives, namely, 2·3 per cent. in the group 16 years and over to 17 per cent. in the under 2 years group (unpublished reports 3rd Medical Laboratory, U.S. Forces), one might expect a fairly high infection rate among the mosquitos. Actually, however, the sporozoite rate was low. In 1,737 *A. punctulatus farauti* examined, only one gland infection was found, an infection rate of 0·06 per cent. ; and of 200 *A. subpictus* one female was seen with oocysts, a 0·5 per cent. infection rate.

These adults were carefully examined for wing and ovary stages. The findings are given in Table VIII.

TABLE VIII.

Ovary and wing stages—Mirivasi.

Species	No. dissected	Wing stage per cent.					Ovary stage per cent.				
		I	II	III	IV	V	I	II	III	IV	V
A. punctulatus farauti	1,737	52·5	36·5	10·2	0·8	0	0·1	84·7	10·2	3·6	1·4
A. subpictus	263	17·1	41·4	32·2	9·3	0	0	58·2	16·8	9·3	15·7

The mosquitos were brought in by small native boys. They were captured feeding on man, dog or pig, or resting on the walls of huts. Most of them were therefore in various stages of engorgement, and no information was gained regarding their ages in relation to this factor. They were not examined until the day after capture, which accounts for the very small percentage of Stage I ovaries. By far the majority of *A. punctulatus farauti* had Stage II ovaries and the very small numbers with Stage IV and V indicate that only a few of the captured adults had had a previous blood meal. The percentage in the case of *A. subpictus* was much higher, evidence that this species was longer lived, or had different habits (*cf. A. amictus hilli* at Cairns).

Similarly, the wing stages indicate that the *A. subpictus* populations probably included a higher percentage of older females. On the whole, therefore, it seems possible that the low infection rate among *A. punctulatus farauti* was due to a short-lived population, the season of the year, when no rain had fallen for several months, together with the strong, south-easterly winds, rendering conditions unfavourable for longevity. *A. subpictus* appears to be able to survive these conditions better

Discussion.

A. punctulatus.

The available evidence indicates that the habits of wild adults of both of the subspecies and the intermediate forms of *A. punctulatus* have much in common. In the first place, it is apparent that the females disperse from their breeding grounds shortly after emergence, and tend to concentrate around a source of blood-meals, such as a human settlement. At Cairns, large numbers of *A. punctulatus farauti* were caught in the open at night. In New Guinea on the other hand, this method was unsuccessful, and adults were obtained in numbers only when the catching station was established within a human settlement. It must be remembered, however, that at Cairns, the breeding area, the source of blood and the daytime resting places were all within the one area, whereas in New Guinea, the habitations were small and scattered, and were located some distance away from the more prolific breeding grounds.

At Cairns, also, the number of *A. punctulatus farauti* entering brightly lighted rooms in houses built several feet off the ground was very small in comparison to the number hunting in the open, whilst in New Guinea dimly lit huts built on the ground proved a prolific source of females. Whilst this behaviour may be due partly to a negative phototropism, it is considered that it is mainly associated with a habit of hunting close to the ground. This opinion is supported by the preference of the females for the lower parts of the body.

Whilst occasional females may attempt to bite during daylight, as, for example, when disturbed in the daytime resting places during dull, showery weather, activity does not usually commence until just after dark. In the localities studied no evidence could be found in three all-night catches of any marked periodicity at dawn and dusk as has been widely held. There appears to be a gradual increase in numbers during the pre-midnight hours, the insects becoming most abundant during the early hours of the morning, thereafter to disappear at daylight.

The fact that females may rest, sometimes for over an hour, in between periods of activity when seeking a blood-meal, indicates that these mosquitos do not make long continuous flights. They are capable, however, of dispersing from the breeding grounds, at least one mile in the case of the female, and at least 600 yards in the case of the male.

After feeding, the engorged female usually rests close to its host, and may not move from there for up to an hour. In most cases, it then flies outdoors, and seeks a suitable habitat in the bush to digest its blood-meal. These bush diurnal resting places occur in moist, sheltered spots, usually close to the ground, and in the vicinity of the source of blood. Here may be found newly emerged females awaiting their first blood-meal, females newly fed or with the blood-meal in various stages of digestion and females that have recently oviposited and returned to shelter. The presence also of large numbers of males in these habitats strongly indicates that fertilisation of the female takes place here and not in the breeding area. Thus, it would appear that, after emerging, the males spend their lifetime in the bush habitat, whilst the females leave it only for the functions of feeding and oviposition.

For the most part, the number of Anophelines which are found indoors during the day is very small, but occasionally one finds many present. At Mirivasi, for example, they were fairly plentiful in huts, which were small and dark, without windows, and with closed doors. In this instance, we attributed the numbers to the fact that this type of hut acted as a trap, the mosquitos finding it difficult to escape. At Nadzab, the daily indoor figure attained high proportions. The huts, here, were built with an overhanging roof, sides ending several feet above the ground and earth floors. Anophelines were found in numbers, not only in such sheltered positions as depressions in the earthen floor, in boxes, among clothes and beneath the sleeping platform, but also in exposed places on the walls and roof. At the time of our visit, and also when seen by Woodhill (1946) several weeks previously, the indoor population was composed entirely of females that had fed the previous night. There were no unfed females or males, such as might be expected if the species normally rested indoors in the daytime in this area. One is therefore inclined to believe that the number of fed females found in these huts during the day is simply an indication of the very large Anopheline population present in the vicinity. It also seems possible that the engorged females found indoors represent those that had fed very late in the night, and were prevented from moving out into the jungle by the onset of daylight.

The daytime resting habits have a very important bearing on adult control. Species which shelter in huts during the day, such as *A. minimus*, Theo., in Assam and North Bengal (Thompson, 1941), *A. culicifacies*, Giles, and the *A. fluviatilis*, James, group in India (Senior White, 1945) and especially *A. gambiae*, Giles, and *A. funestus*, Giles, in Africa (Haddow, 1942), can be controlled by spraying the huts with pyrethrum sprays. It is obvious that pyrethrum sprays applied indoors during the day would have little effect on *A. punctulatus*. DDT sprays applied to hut walls, however, are much more promising against this species, because of its habit of resting on the walls before and after feeding. Indeed, encouraging results with this method of control have been obtained by the authors at Salamaua, and, in co-operation with the 3rd Medical Laboratory, U.S. Forces, at Mirivasi (unpublished U.S. Army report).

The information regarding zoophilous or androphilous preference of *A. punctulatus farauti* indicates that, if any preference exists, it is not very pronounced, and that this subspecies may simply feed on the nearest available host, be it domestic animal, bird or man. In the case of *A. punctulatus punctulatus*, androphilism appears to be very marked, for, in a series of 87 gut samples collected by Bayley and Aberdeen from females in outdoor resting places near native villages at Milne Bay, no less than 94 per cent. were positive for man (unpublished Army reports).

The final point in reference to the Salamaua observations is the very marked preference the Anophelines displayed for the native, a feature which could be given consideration in the location of native compounds in relation to white settlements and Anopheline breeding grounds.

A. amictus hilli.

The habits of *A. amictus hilli* have much in common with those of *A. punctulatus*. There are, however, some outstanding differences. At Cairns, large numbers of both males and females of *A. amictus hilli* were found in the immediate vicinity of the breeding grounds (*cf. A. punctulatus farauti*). Only a very small percentage of these were fresh emergences, or had recently had a blood-meal. Interpretations of the ovary development in the females indicated that the majority were there for the purposes of oviposition. Those females with Stage II follicles had deposited their eggs, and those with Stage V follicles were about to do so. Visits to this swamp during the day at a later period, when breeding was not occurring there, but whilst adults were still fairly prevalent at night in the vicinity, failed to reveal any males or females. This evidence confirms the opinion that the females found resting on and around the swamp were there for oviposition and not merely for shelter.

The large numbers of males observed during the day on the breeding area is a clear indication that fertilisation of the female occurs here and not, as in the case of *A. punctulatus farauti*, in the bush.

The favoured daytime resting place of those females that are awaiting a blood-meal and those that have recently fed still awaits determination. Eight females were found indoors, and eleven in an outdoor habitat similar to that used by *A. punctulatus farauti*. Of the 19 taken, 14 had fed the night prior to capture and four on some previous night. It is interesting to compare the numbers of the two species taken in the outdoor habitat during the period of the investigation, namely 11 *A. amictus hilli* and 211 *A. punctulatus farauti*.

Nothing is known of any host preference by *A. amictus hilli*, the few blood spots collected all having been from females taken in houses. In certain localities on the North Queensland coast, however, it is a vicious pest, and at Cairns it showed no hesitation in attacking the members of the catching team. Reports from northern mangrove swamps, where it occurs in pest proportions—Port Stewart for example—indicate that it can be fairly active during the day as well as at night. Our experience at Cairns does not confirm this, and, in a single all-night catch, the females did not appear until just after dark, and then attacked in waves, with a small peak of abundance from 8.30 to 9 p.m.

Other Species.

A considerable amount of work remains to be done on the habits of adult Australasian Anophelines. The information on the species dealt with in this paper is still incomplete, and we know practically nothing of the other species. *A. bancrofti bancrofti*, as mentioned earlier, is very common in the Cairns district. Observations by Heydon (unpublished) and the authors show that here it rarely attacks man, and, although susceptible to experimental infection with both *Plasmodium falciparum* and *P. vivax* (Heydon, unpublished ; Mackerras and Roberts, in the press) is not regarded as a vector of any importance. In the Northern Territory, however, (Lee and Woodhill, 1944), and in Dutch New Guinea at Merauke and Tanah Merah, it is a vicious pest, and at Tanah Merah was found by De Rook (quoted from Lee and Woodhill, 1944) to have an infection rate of 4·3 per cent.

A. annulipes, Walker, is an extremely common species, well distributed throughout Australia, and extending into New Guinea. It is highly susceptible to experimental infection with *P. falciparum* and *P. vivax* (Roberts, 1943), and, until Heydon's determination of *A. punctulatus farauti* as the natural vector of *P. vivax* in North Queensland, had been held responsible for all outbreaks of malaria. On epidemiological grounds, it is now considered as the probable vector in sporadic cases of malaria in areas outside the distribution of *A. punctulatus farauti*. Of its adult behaviour, Lee and Woodhill (1944) say : " In general, females feed readily on man, especially after sunset, and in the Northern Territory tend to remain in tents and huts on the day following the blood feed." Our experience with this species is by no means extensive, but we have found that at night it will attack man viciously in the vicinity of its breeding area, and will readily enter houses and tents to feed. We have seen it resting during the day in tents, slit trenches, and on the banks of creeks in much the same type of habitat as is favoured by *A. punctulatus farauti.*

Summary.

Observations are recorded on the habits and behaviour of wild adult *A. amictus hilli* and *A. punctulatus farauti* at Cairns, North Queensland, and of the two subspecies and intermediate forms of *A. punctulatus* in New Guinea.

The most important points determined, which bear on the control of adults of *A. punctulatus*, are :—

(1) Females disperse from the breeding area shortly after emergence. During the day, they are rarely found indoors in any numbers, but hide away in moist, sheltered places in the bush in the vicinity of the source of the blood-meal. Fertilisation of the female takes place here, and these diurnal resting places are vacated only for the purposes of feeding and oviposition.

(2) Both before and after feeding, females rest on the walls, etc., of huts and houses, which they readily enter. There is some evidence that the normal flight is close to the ground, and only comparatively small numbers enter houses built several feet off the ground.

(3) Activity does not usually commence until just after dark, and the peak of abundance occurs in the early hours of the morning.

(4) Females may disperse at least one mile from their breeding grounds in search of a blood-meal.

(5) In New Guinea there is a distinct preference for the native.

(6) Whilst *A. punctulatus farauti* does not show any marked preference for man, animal or bird, *A. punctulatus punctulatus* has very definite androphilous tendencies.

The habits of *A. amictus hilli* are broadly similar to those of *A. punctulatus farauti*, but differ in important details. It does not rest indoors during the day, but its favoured outdoor habitat awaits determination. Only a few females were found at Cairns in the bush diurnal resting places used by *A. punctulatus farauti*, whilst the large numbers observed on the breeding area were there for the purposes of oviposition. Fertilisation takes place in the breeding area, where the males remain. There also appears to be a different nocturnal periodicity from that observed for *A. punctulatus farauti*.

An attempt was made to determine the ages of the females captured at night and during the day by means of the criteria of ovary development, wing condition, presence of blood and of spermatozoa. The interpretation of these criteria was assisted by observations on bred *A. punctulatus punctulatus*.

Acknowledgement.

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References.

Christophers, S. R., Sinton, J. A. & Covell, G. (1944). How to do a malaria survey. 4th edn.—Hlth Bull. no. 4 (Malar. Bur. no. 6). 156 pp.

Haddow, A. J. (1942). The mosquito fauna and climate of native huts at Kisumu, Kenya.—Bull. ent. Res., **33**, pp. 91–142.

Heydon, G. A. M. (1923). Malaria at Rabaul.—Med. J. Aust., 15 Dec., 1923, pp. 625–633.

Lee, D. J. & Woodhill, A. R. (1944). The Anopheline mosquitoes of the Australasian Region. — Monogr. Dep. Zool. Univ. Sydney, no. 2, xii, 209 pp.

Roberts, F. H. S. (1943). Observations on *Anopheles annulipes* Walk. as a possible vector of malaria. I. The relative susceptibility of *A. annulipes* and *A. punctulatus* var. *moluccensis* Sw. to experimental infection with malaria parasites.—Aust. J. exp. Biol. med. Sci., **21**, pp. 259–262.

Russell, P. F. (1932). Daytime resting places of Anopheline mosquitoes in the Philippines.—Proc. ent. Soc. Wash., **34**, pp. 129–138.

Senior White, R. (1945). House spraying with DDT and pyrethrum extract compared.—J. Malar. Inst. India, **6**, pp. 83–87.

SWELLENGREBEL, N. H. & RODENWALDT, E. (1932). Die Anophelen von Niederländisch-Ostindien.—Jena, G. Fischer, 242 pp.

THOMPSON, R. C. Muirhead. (1941). Studies on the behaviour of *Anopheles minimus*. Part V. The behaviour of adults in relation to feeding and resting in houses.—J. Malar. Inst. India, **4**, pp. 217–245.

WOODHILL, A. R. (1946). Observations on the morphology and biology of the subspecies of *Anopheles punctulatus* Dönitz.—Proc. Linn. Soc. N.S.W., **70**, pp. 276–287.

TOXICITY OF LIMEWASH CONTAINING DDT OR "GAMMEXANE" TO MOSQUITOS (*AËDES AEGYPTI*, L.).

By J. Hadjinikolau and J. R. Busvine.

Entomology Department, London School of Hygiene and Tropical Medicine.

Residual insecticides have shown much promise in reducing the mosquito population inside houses over long periods. It has been hoped that this might prove a valuable aid in controlling malaria, though it appears that much depends on the insecticides being lethal to the mosquitos after fairly short periods of contact (see Muirhead Thomson, 1947).

In many countries, especially in country districts, the houses have plaster or mud walls brightened and decorated with a coating of limewash. It would be a very simple matter for a solid insecticide such as DDT to be incorporated with the wash and applied in this way each season. The purpose of these experiments was to obtain a preliminary impression as to whether this method would be feasible.

Biological Material.

The mosquitos used throughout were *Aëdes aegypti*, L., because they were available in large numbers in laboratory cultures throughout the year. Young adults 2–5 days old were taken. The stocks were reared as described by David and others (1944) and the adults kept in a constant temperature room at 26°C. The experiments were conducted at room temperature (18–23°C.) and each batch of mosquitos was brought into the laboratory several hours before testing to give them time to acclimatise.

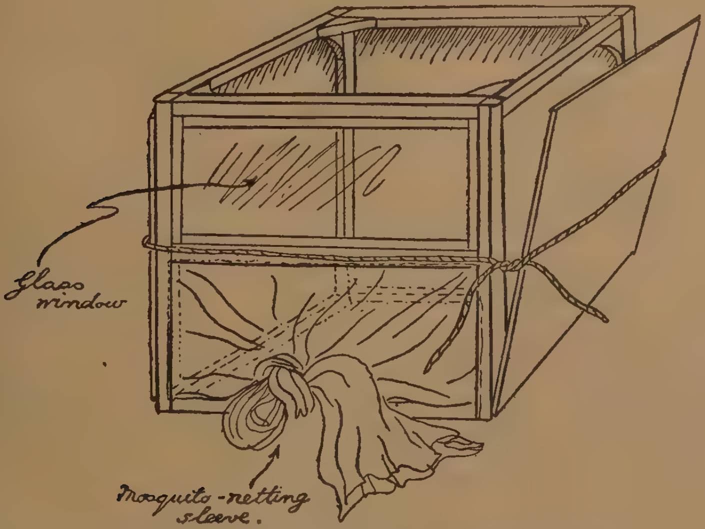

Fig. 1.—Test cage partly assembled to show construction.

Apparatus.

Exposures to the insecticides were made in small cages of the type shown in fig. 1. Three detachable walls and a ceiling of Essex board were applied to a framework of ½-inch wooden lathes. The fourth wall comprised a window and a "sleeve" of mosquito netting for introducing and extracting the mosquitos. Essex boards were chosen to simulate walls with a very absorbent surface. As the walls were interchangeable, it was possible to test a variety of treatments with a small number of cages.

Application of Insecticide.

The insecticides were made up either in "whitewash" or in kerosene solution, the former being applied by brush and the latter by dripping the liquid as evenly as possible from a pipette at the rate of 10 cc. per square foot.

The whitewashes consisted of about 1·6 gm. lime and 6 cc. of water per square foot. The appropriate quantity of DDT (an 85 per cent. grade obtained from the Geigy Co. Ltd.) was mixed in with the aid of a small quantity (1 per cent.) of Teepol, a wetting agent supplied by Messrs. Shell Chemicals Ltd. "Gammexane" was added in the form of a dispersible powder containing 5 per cent. active principle (P530 supplied by Messrs. Imperial Chemical Industries Ltd.).

Procedure.

Approximately one hundred mosquitos were introduced into each cage and collected again at the end of the exposure by means of a sucking tube. They were then blown into clean cages of similar design which were kept humid by damp cloths hung over the aperture carrying the mosquito netting sleeve. The insects were examined at intervals and mortality counts of each sex determined on the day after exposure.

Observations.

During the exposures, the mosquitos were free to fly or to settle on the treated or untreated parts of the cage. The areas of various portions of the cage were :—

Three treated walls and ceiling : total ...	3·6 sq. ft.
Untreated floor	0·85 „ „
„ glass window and sleeve ...	0·85 „ „
„ wooden battens	0·67 „ „

The insects seldom rested on the window or sleeve but often settled on the wooden framework of the cage. Owing to the irritant effect of DDT (Kennedy, 1947) the mosquitos in DDT-treated cages appeared to spend more time in flight and a larger proportion rested on the untreated framework than in untreated control cages. It was not clear whether this was equally true with P530 ; in any case, the insects developed signs of intoxication fairly rapidly with this insecticide.

It was noticed that after being confined for a short time in DDT-treated cages, the mosquitos displayed restlessness as described by Kennedy (1947). After an exposure of one or two hours all the insects would show the characteristic signs of DDT poisoning. If they were then removed to a clean cage, a proportion of them would usually recover by next day ; and if offered a blood-meal, some would feed and lay viable eggs. Thus, mosquitos can recover from mild DDT intoxication.

Results.

(*a*) *Variability in resistance.*—Owing to fluctuations in resistance of different batches of mosquitos and the rather rough nature of the test method, the results were somewhat variable. Table I shows the mortalities produced by a standard exposure in three cages and a control on different dates. Some of the inconsistency may have been due to changes in room temperature and to different periods allowed for acclimatisation.

TABLE I.

Variations in kills of *A. aegypti* resulting from two-hour exposures in cages treated with DDT.

Dates of Exposure	Percentage kill of mosquitos by 2-hour exposure (Dose in mg. DDT per sq. ft.)							
	Treatments 25.iii.46						Control	
	Whitewash				Kerosene solution			
	80 mg. DDT		160 mg. DDT		160 mg. DDT			
	♂'s	♀'s	♂'s	♀'s	♂'s	♀'s	♂'s	♀'s
5.iv.45	99	100	93	100	71	48	0	0
8.iv.46	93	80	100	95	96	92	0	0
11.iv.46	30	35	88	77	62	60	5	2
15.iv.46	44	36	98	97	42	42	12	8
9. v.46	100	63	100	90	100	41	12	3
24. v.46	96	97	100	100	72	65	0	0

(*b*) *Mortality and exposure time.*—Table II shows the mortalities recorded after different exposures in cages treated with DDT. It will be found that the figures are reasonably consistent with the logarithmic-sigmoid curves typical of dosage-mortality relations.

TABLE II.

Relation of mortality to the period for which the mosquitos were confined in the treated cages.

Exposures	Percentage kill of mosquitos (Dose in mg. DDT per sq. ft.)					
	Treatments					
	Whitewash				Kerosene solution	
	80 mg.		160 mg.		160 mg.	
	♂'s	♀'s	♂'s	♀'s	♂'s	♀'s
1 hour	56	40	90	67	7	0
2 hours	69	52	89	85	53	53
3 ,,	100	100	100	100	76	97
4 ,,	96	93	100	100	96	78
5 ,,	100	100	100	100	78	94

Males were generally rather more susceptible than females.

(*c*) *Method of application of insecticides.*—It is clear from Table II that in these experiments the DDT in whitewash was more effective than a DDT-kerosene solution. The kerosene treatment, however, was decidedly handicapped by the method of application by pipette which required an unusually large volume of liquid tending to cause deep penetration of the insecticide. At the time of these experiments, no suitable apparatus was available for applying very light standard deposits evenly over areas of the required size.

(*d*) *DDT versus " Gammexane".*—The first entries in Table III give comparable results for P530. These show the much higher toxicity of this insecticide to *A. aegypti* when applied in this form. It appears to be more than equally effective at one-tenth the dose and much more rapid in action.

(*e*) *Persistence of the insecticides.*—A few observations were made on the persistence of the insecticidal treatment by tests with Essex boards at various intervals after application. These results are set out in Tables III and IV.

TABLE III.

Toxicity of " Gammexane"-treated cages to *A. aegypti* at different intervals after application.

Interval between treatment and exposure	Percentage kill of mosquitos by " Gammexane "-treated cages (Dose mg. gamma isomer per sq. ft.)											
	Treatments											
	½ hour's exposure						1 hour's exposure					
	Whitewash				Kerosene		Whitewash				Kerosene	
	8 mg.		40 mg.		40 mg.		8 mg.		40 mg.		40 mg.	
	♂'s	♀'s	♂'s	♀'s	♂'s	♀'s	♂'s	♀'s	♂'s	♀'s	♂'s	♀'s
1 week	25	16	100	100	100	100	94	100	100	100	100	100
6 weeks	—	—	100	100	100	100	95	87	—	—	—	—
10 ,,	29	50	100	100	100	100	41	17	—	—	—	—
13 ,,	—	—	100	100	100	100	57	23	100	100	100	100
22 ,,	—	—	93	66	100	97	—	—	100	100	100	100

TABLE IV.

Toxicity of DDT-treated cages to *A. aegypti* at different intervals after application.

Interval between treatment and exposure	Percentage kill of mosquitos by 2-hr. exposure in DDT-treated cages (Dose mg. DDT per sq. ft.)					
	Treatments					
	Whitewash				Kerosene solution	
	80 mg.		160 mg.		160 mg.	
	♂'s	♀'s	♂'s	♀'s	♂'s	♀'s
1 week	100	100	100	100	77	48
8 weeks	97	97	100	100	70	65
7 months	9	7	49	34	21	26

There seems to be a remarkable degree of persistence in spite of the fact that limewash has been reported as being chemically detrimental to DDT and "Gammexane". Fleck & Haller (1944), however, found no dehydro-chlorination of DDT by calcium oxide even at 115°–120°C. for one hour. Possibly the adverse effect in some samples may be due to impurities such as iron compounds, some of which seem to have a catalytic action in hydrolysing DDT.

Summary.

Limewashes containing DDT or "Gammexane" were tested for insecticidal action on mosquitos (*Aëdes aegypti*) by confining the latter in cages with treated walls and ceilings. Only about 60 per cent. of the total internal surface of the cages were treated but the mosquitos were averse to settling on the floor, window, etc., which constituted a large part (28 per cent.) of the untreated area.

Limewashes containing 160 mg. DDT per square foot gave a high kill of mosquitos after two hours' exposure in the cage; and 8 mg. per square foot of the active principle of "Gammexane" gave a high kill with a one hour exposure.

Both treatments remained effective for 6–8 weeks under temperate conditions.

References.

David, W. A. L., Bracey, P. & Harvey, A. (1944). Bull. ent. Res., **35,** p. 227.

Fleck, E. E. & Haller, H. L. (1944). J. Amer. chem. Soc., **66,** p. 2095.

Kennedy, J. S. (1947). Bull. ent. Res., **37,** p. 593.

Muirhead Thomson, R. C. (1947). Trans. roy. Soc. trop. Med. Hyg., **40,** p. 511.

THE MOSQUITOES OF BWAMBA COUNTY, UGANDA.
VI.—MOSQUITO BREEDING IN PLANT AXILS.

By A. J. Haddow.*

From the Yellow Fever Research Institute, Entebbe, Uganda.†

(Plates XIII–XX.)

As has been explained in previous communications (Haddow, 1945 a, b, c ; 1946), field studies of mosquitoes have been carried out in Bwamba County, Uganda, since the beginning of 1942, having been undertaken because yellow fever virus had been isolated in 1941 from a human patient in that area and from wild-caught *Aëdes* (*Stegomyia*) *simpsoni*, Theo., which was incriminated (1941) as the principal local vector of human yellow fever (Mahaffy and others, 1942). An efficient mass-vaccination of the entire population of Bwamba was carried out in 1941, but subsequent studies (Haddow and others, 1947) showed that yellow fever was endemic among monkeys of the lowland forest, and two additional strains of yellow fever virus were isolated from mosquitoes, the first from *A. simpsoni* taken in native plantations in 1942 and the second from *Aëdes* spp. (not including *A. simpsoni* or *A. aegypti*, L.) taken in uninhabited rain forest in 1944 (Smithburn and Haddow, 1946). Most of the field work has been devoted to a search for the forest vector responsible for the maintenance of the animal-to-animal cycle, but observations on *A. simpsoni* have also been carried out whenever possible, and the results of some of these—concerning the biting habits of the adult mosquito—have been published (Haddow, 1945 b, c). The purpose of the present paper is to discuss the results obtained by a study of plant axils as breeding places for mosquitoes, with special reference to *A. simpsoni*, which is by far the most important axil-breeder in Bwamba.

Bwamba County is in the extreme west of Uganda, lying between the Semliki River and the north spur of Ruwenzori (Mountains of the Moon). The region has been described elsewhere (Haddow, 1945 a) but some information on the inhabited sections must be given here. Much of the area is covered by dense virgin rain forest, which forms a belt 6 to 7 miles wide along the Semliki River. This section—the Semliki Forest—is a Crown reserve and no settlement is permitted within its limits. It represents the most easterly extension of the great Central African rain forest, or Ituri Forest, and its fauna is essentially " West African " in type. The other main forest area of Bwamba is the Ruwenzori Forest—also a Crown reserve—which has a lower limit marked by a cut line at about 7,000 feet in the north spur of the mountain. Between these large forests lies a fairly densely inhabited agricultural zone, with a population of over 30,000 Africans. This cultivated area shows great diversity, extending as it does from warm humid forest at an altitude of about 3,000 feet to exposed and windy grasslands at about 7,000 feet. There are many small streams, most of them fringed by relict strips of primary forest ; and much of the cultivation is carried on by the African community on the ridges between their deep valleys. In a few places the valley floors are wide enough to allow the establishment of riverside plantations. The warm climate and well-distributed rainfall of 50–60 inches per year, which characterise the lowlands of the county, permit the successful cultivation of a considerable variety of food and cash crops, while a rather different group is produced on the wet but exposed mountain slopes.

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* Staff Member of the International Health Division, Rockefeller Foundation.

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In the lowlands the main cash crops are coffee, cotton and the recently introduced "dry rice," which last is taking an increasingly important place in the local diet and seems to be replacing bananas in many areas. The most important food crops are bananas of many kinds, cassava, sweet potatoes, maize and what may be referred to collectively as "colocasias" (*Colocasia esculentum* Schott. and *Xanthosoma sagittifolium* Schott.). Groundnuts, beans, pineapples and simsim (*Sesamum*) are raised on a smaller scale. On the mountain slopes bananas are grown in the more sheltered localities but are of less importance than in the lowlands, most of the available land being given over to millet and beans. *C. esculentum* is grown quite extensively in the mountain valleys.

Native agriculture is largely based on the usual wasteful shifting system, and is almost always of a mixed nature. It is quite exceptional to find a holding given over exclusively to a single crop. Most families cultivate a banana plantation (including several varieties) with a heavy undergrowth of colocasias and often with a few pineapples. As will be shown below, these mixed plantations are the main breeding places of *A. simpsoni.*

The late Mr. E. G. Gibbins carried out studies on axil-breeding mosquitoes in Bwamba during 2 months of 1941, but only his main findings were known to the writer when work was begun in 1942. This resulted in a certain amount of duplication in the first few months which, however, served to confirm many—though not all—of Gibbins' findings. The main information published by Gibbins (1942) was that the principal sources of *A. simpsoni* in Uganda were the "gonja" group of bananas or plantains, colocasias and pineapples. It should be recorded that the discovery of the importance of the "gonja" group was made by Mr. J. D. Gillett, who also collected a considerable proportion of the data discussed by Gibbins.

Before proceeding to a discussion of the plants studied and of the mosquito larvae found in their axils, it may be appropriate to summarise briefly what is known of the habits of the adult *A. simpsoni*, the only important axil-breeder in Bwamba. In Uganda this species oviposits almost exclusively in plant axils, though in other parts of Africa it appears to breed somewhat extensively in tree-holes (Dunn, 1926 ; 1927). Hopkins (1936) mentions the scarcity of the larvae in tree-holes in Uganda and the writer, in a survey of 300 tree-holes in Bwamba, found only three *A. simpsoni* larvae among a total of 2,147 larvae. No larvae of the species were found in a survey of 100 tree-holes in the Ruwenzori Forest (1,828 larvae) or in a survey of a similar number in the Kibale Forest in Toro (1,481 larvae). In the Entebbe area, where several hundred tree-holes have been examined, this species also seems to oviposit exclusively in axils. The adults may be regarded as "semi-domestic" in that they attain their maximum concentration among crops, though they seldom or never enter huts. They abound in banana plantations, the greatest numbers occurring in the thinner parts and round the edges. From these plantations they disperse into adjacent maize fields, cassava plantations and coffee gardens. They penetrate only the extreme edges of forest (and that in very small numbers) even when extensive breeding grounds are actually contiguous with the forest edge. *A. simpsoni* is almost exclusively diurnal, biting throughout the day, but attaining its highest peaks of activity in the morning and late afternoon. It is most active in warm humid weather. It seems to prefer human blood, and attacks the head and shoulders of its victim selectively, no matter what the posture of the host may be (Gibbins, 1942 ; Haddow, 1945 b, c).

The Plants Studied.

Gibbins, during his work in Bwamba, studied various types of banana, colocasias, pineapple, *Sansevieria* spp. and *Dracaena ugandensis.* He also made some observations on the wild banana and *Dracaena steudneri.* During the writer's preliminary survey, samples were collected from 3 types of banana, colocasias, pineapple and *Dracaena* *ugandensis*. In later work the list was extended to include the wild banana, wild and garden canna, *Sansevieria* spp., the colocasia of the mountain slopes, *D. steudneri* and the screw pine (*Pandanus chiliocarpus*). The general characteristics of these plants are as follows :—

1. *The Wild Banana.*

In Bwamba the wild banana, *Musa* (*Physocaulis*) sp. indet., is confined to the lower borders of the Ruwenzori Forest at an altitude of 6,500 to 7,500 feet. The leaves spring in an enormous rosette almost from ground level, and even in an old plant there is no real " stem," the highest leaves taking origin only some 5 to 6 feet above ground. The individual leaves are extremely large (Pl. XIII, fig. 1), sometimes reaching a length of 15 feet and a breadth of 3 feet or more. To a large extent they remain entire and do not become tattered like those of the cultivated bananas. The numerous huge axils of the wild banana hold considerable quantities of water and harbour exceptionally large numbers of larvae. Africans crossing the mountain passes in wet weather lop off the outer leaves of this plant, which is known locally as "kitembe," to serve as umbrellas and to make temporary shelters. The small fruit is not used as food in Bwamba.

2. *The Cultivated Bananas.*

Numerous varieties of banana are grown in Uganda, and it has been the custom to subdivide these into bananas proper, which are eaten raw, and plantains, which are gathered unripe and cooked. This distinction seems to the writer to be rather artificial, particularly when one considers the important gonja group, which may be eaten raw when fully ripe or roasted when half ripe. Further, Thomas (in Tothill, 1940) points out that the formerly accepted morphological distinctions between the plantain group, *Musa* (*Eumusa*) *paradisiaca*, and the banana group, *Musa* (*Eumusa*) *sapientum*, are not reliable, none of the characters being constant, at least in so far as Uganda is concerned. It therefore seems best to follow this worker, who employs the grouping used by the African consumers themselves. Of the four main groups recognised by Thomas, only three—bitoke, menvu and gonja—are of importance in Bwamba. As considerable variations occur within these groups in different parts of Uganda, the following description should be taken as applying essentially to the commoner Bwamba types.

The bitoke bananas, which are locally known under the collective name " namunu," form one of the main foods of the county and are thus of considerable importance. The fruit is gathered unripe, and the adherent skin is scraped off with a knife, after which the bananas are steamed or boiled and are eaten whole or pounded. Certain varieties are used in making beer. The plants (Pl. XIII, fig. 2) are tall —usually 10 to 12 feet to the leaf bases—and the dark-green leaves are rather long and slender, with green or pinkish midribs. Among the Bwamba varieties the stems are generally green in the upper part, with pronounced black blotches (fig. 1). In general the withered leaves rot quickly and drop from the plants, and there remain only the extreme bases of the shrivelled petioles sheathing the lower part of the stem. In cross-section the petioles form very shallow open gutters, the edges of which are everted in the lower part near the main stem. As a result the bases of the petioles grip the stem very loosely and the axils are far from watertight. The tendency of the large lower leaves to droop outward opens the axils still further. The angle between successive pairs of leaves (as seen in a cross-section through the crown of the plant) is very large—about 45°—and this also results in the formation of very wide, loose axils. Except immediately after heavy rain it is only in the topmost axils, which have not yet completely opened, and in the rolled topmost leaf that water is able to persist in significant amounts.

The menvu group (Pl. XIV, fig. 1) consists of small or medium-sized sweet bananas with yellow or rufous skin, known locally as " kasukali " and " kitoke kyekijungu," respectively. The fruits are usually eaten raw but are sometimes boiled or steamed after partial sun-drying. In Bwamba they are extensively used in making beer. In general, the plants are tall—10 to 15 feet—and the rather light green leaves are more erect and more rounded at the tips than in the case of the bitoke group. The withered leaves are often very persistent, and the bases of their petioles frequently form a thick fibrous sheath round the main stem. The stem, which is best seen just below the leaves, is seldom or never of the fresh green which characterises bitoke plants. It is generally buff or pinkish in colour and the blotches are dark brown and relatively limited in extent, in contrast to the large glossy-black markings of the bitoke group. The petioles, which are pinkish-buff in the "kasukali" group and deep pink in the "kitoke kyekijungu" group, are very long, slender and rather stiff. Apart from this they closely resemble the bitoke petioles, having a very open dorsal gutter and edges which are markedly everted near the main stem (fig. 1). Thus, as in the case of the bitoke bananas, the axils are very open and far from watertight. Once again the angle between successive pairs of leaves is large. Occasionally, in young plants five to six feet high, the petioles grasp the main stem tightly, and in these cases the axils may retain a fair amount of water.

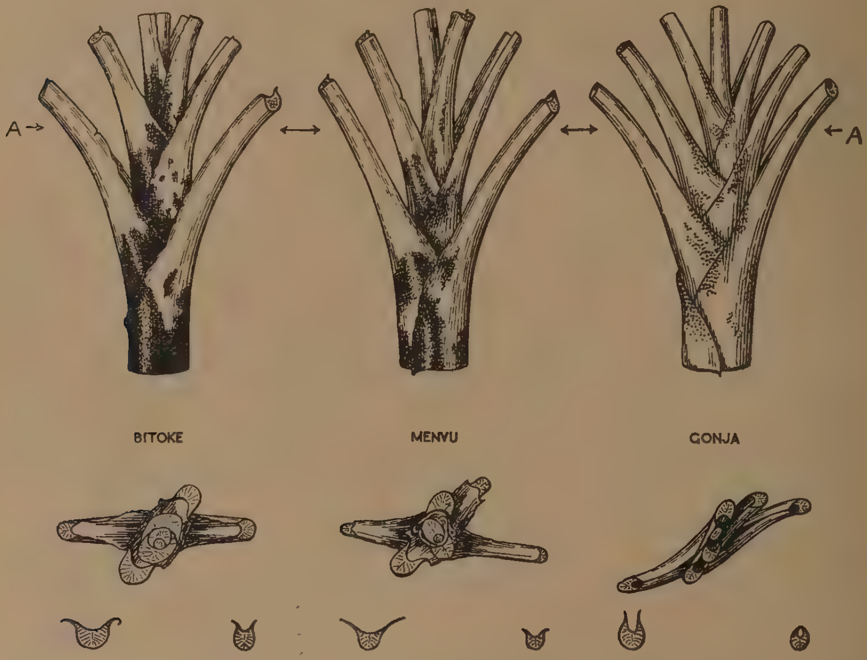

Fig. 1.—The leaf-bases of the 3 main types of banana grown in Bwamba—bitoke ("nkobe" variety), menvu ("kitoke kyekijungu" variety) and gonja ("kajabo" variety). Note the dark markings of the bitoke and menvu bananas and the closer structure and more numerous petioles of the gonja. The sections through the crown show the angle between successive pairs of petioles and the sections of the petioles themselves show the form of the dorsal gutter at two points.

The gonja group (locally known as "kikonje") produces very large sweet bananas, in some cases as long as a man's arm, and is of great importance in relation to

A. simpsoni. Most plantations in Bwamba include at least a few gonjas, as these bananas are used, mixed with other types, in making the favourite local beer. They are also roasted in the skins when partly ripe or eaten raw when fully ripe. The plants (Pl. XIV, fig. 2) are large when full grown, usually measuring about 12 feet to the lowest leaf bases. The leaves, which are of a rather light green with greenish midribs, stand up much more stiffly than do those of other types. The bases of the withered petioles, as in the case of the menvu group, tend to form a dry fibrous outer coat to the main stem, which is buff or pinkish-buff in colour, with only a few faint brown markings near the bases of the petioles. The reason for the importance of this group is to be found in the structure of the petioles themselves. These are rather stiff and slender, with the edges so inrolled that the dorsal gutter is almost closed in except at the base near the main stem. They thus form almost perfect waterways leading down from the large collecting-surfaces of the leaves to the deep axils. Even at the bases of the petioles the edges are slightly inturned and grasp the main stem firmly, with the result that the axils are almost completely watertight. The whole crown of the gonja is much more compact than the crowns of other groups, the petioles rising in very close succession, though the number of leaves is large. A cross-section through the leaf bases (fig. 1) shows that the angle between successive pairs of leaves is small—only about 15 to 20°—with the result that the petioles spring in a close spiral from either side of the main stem, each shading the last to some extent. A large percentage of the axils of a full-grown gonja are capable of retaining water, and the small size of the entry lessens evaporation. For these reasons this group of bananas is much favoured by *A. simpsoni.*

3. *The Colocasias.*

In Bwamba colocasias, or cocoa-yams, whose axils provide a peculiarly suitable breeding ground for *A. simpsoni*, are extensively cultivated. Gibbins (1942) stated that they are generally grown in patches in the open, but the writer has found that they are at least as commonly cultivated as a subsidiary crop in banana plantations, where they form a dense undergrowth.

The species mainly grown in the lowland country is the large *Xanthosoma sagittifolium* Schott. (Pl. XV, fig. 1) which has enormous hastate leaves, sometimes as much as 3 feet long by 2 wide (Pl. XV, fig. 2). It should be noted that the angles of the leaf are clearly marked and that the petiole joins the leaf at the margin. In general these plants grow about waist high, but occasionally large specimens may reach a height of five to six feet. *Xanthosoma* is propagated by cuttings, which consist of the base of the stem and the upper part of the rhizome. There seems to be no special planting season, but the periods just before the long and the short rains are considered the most favourable. Under good conditions, as many as three crops may be taken from a single plot within a year. Both rhizome and leaf are used as food.

The petioles of colocasias are entire in the distal part but basally are grooved deeply (fig. 2). The groove is bordered by thin vertical lips which meet distally and so prevent water from draining to the axils via the petiole. Further, the leaf-surfaces, even in young plants, droop outward at an angle to the petioles, so that their large collecting-surfaces cannot supply water to the axils. Water in colocasia axils must therefore be derived mainly from direct rainfall and from condensation and run-off from the undersides of the stems above the axils concerned. In spite of this apparently meagre catchment area, the axils almost always contain water except in the very driest weather. It should be noted that 2 varieties of *Xanthosoma* occur in the Bwamba lowlands, usually growing side by side. The first, known locally as " binongwe byekijungu," is very important in relation to *A. simpsoni* and may readily be recognised by its fresh grass-green petioles. In the other variety (known as " binongwe byekibutuku ") the petioles are of a very dark smoky blue-green, but as *Xanthosoma* petioles are invariably covered with a bloom of yeasts (like hothouse grapes) in nature they appear dark blue. In this type the lips of the petioles are deep reddish purple. The blue-stemmed variety is less important in relation to *A. simpsoni*, as the lips of the basal grooves are everted slightly (as in the case of bitoke bananas) thus allowing water to leak from the axils, a tendency which is exaggerated by the fact that the large leaves droop outward much more markedly than in the case of the green-stemmed type.

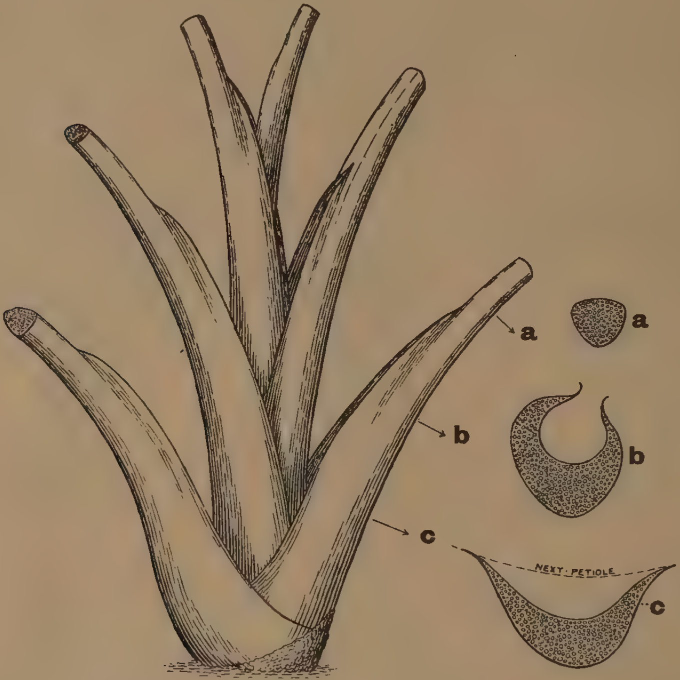

Fig. 2.—The petiole bases of *Xanthosoma sagittifolium* Schott. ("binongwe byekijungu" variety) to show the structure of the dorsal grooves and their marginal lips.

Xanthosoma axils are very deep and rather open above. They are capable of retaining relatively large quantities of water and thus form an excellent habitat for *A. simpsoni* larvae. Being close to the ground they are easy to examine and they admit a pipette easily. It is therefore comparatively simple to draw off the water from *Xanthosoma* axils, whereas in the case of a plant like the gonja it may be necessary to wash out the deep narrow axils with several changes of water to ensure capture of all the larvae, as a pipette fine enough to penetrate to the bottom is too narrow for larva collection. For these reasons the writer regards *Xanthosoma* as the ideal plant for rapid survey or for detailed field investigations.

On the mountain slopes *Colocasia esculentum* Schott. (frequently referred to in literature on Uganda as *C. antiquorum* Schott.) is the species commonly grown (Pl. XVI, fig. 1). This plant, locally known as "binongwe byekikonjo" is usually smaller than *X. sagittifolium* and the leaves are peltate in form, with somewhat rounded angles and curved margins (Pl. XVI, fig. 2). The petiole does not join the leaf at the edge but at a point several inches from it. In *C. esculentum* the petioles are much more slender and flexible than those of the lowland plant and are purplish brown in colour. The axils, though generally smaller than those of *Xanthosoma*, are watertight and favoured by axil-breeding mosquitoes.

4. *Pineapples.*

Though not grown in large quantities in any part of Bwamba, pineapples (*Ananas comosus* Merr.), locally known as "kinanansi," occur almost everywhere in the lowlands, either in small patches in the open or as scattered plants in banana plantations. They are also frequently cultivated in plantations of *Cassia*, *Markhamia* or *Melea*, which are maintained in many places to provide a supply of building poles (Pl. XVII, fig. 1). Very often a few are grown round the hut itself or as a sort of border along the path leading to it.

The majority of Bwamba pineapples are of a small yellow-skinned variety, but the large green-skinned "Queen" type flourishes in one area. The axils are small but well formed, and the great majority of them are capable of retaining water, except where the stem is decumbent. Though the volume of water in each axil is usually small, it is adequate for mosquito development; and furthermore 20 to 30 stems (each with at least 100 axils and a deep terminal cup) may spring from a single root. During the rains a high percentage of these axils will harbour larvae, and the mosquito output of a single large plant in wet weather must be very great.

5. *Sansevieria* spp.

Various species of *Sansevieria*, or bowstring hemp, known locally as "manusi," grow wild in the Semliki Forest and are occasionally cultivated in small casual plots for the sake of the fibre. The mottled succulent leaves are about 2 inches wide and 1 to 4½ feet long (Pl. XVII, fig. 2). Gibbins found the young plants to be fairly favourable breeding places for *A. simpsoni*, but the writer has not found this to be the case in Bwamba. Here, observation has been confined to cultivated plots, as *A. simpsoni* does not occur in the depths of the Semliki Forest where the wild plants grow. Though many of the axils are capable of holding water and though a few do harbour larvae, the great majority (being extremely close to the ground) are so choked with earth and leaf débris as to be useless for mosquito breeding.

6. *Pandanus ? chiliocarpus* Stapf.

In several swampy forest-edge localities the screw pine, *Pandanus*, locally known as "kigagara," forms dense thickets (Pl. XVIII, fig. 1). The larger plants (Pl. XVIII, fig. 2) usually reach a height of about 30 feet, but occasional specimens may greatly exceed this. The stem springs from a pyramid of prop roots, taking its origin 3 to 4 feet above the surface of the semiliquid black mud which seems to be the type of soil preferred. In young plants the lowest branches may be about 6 feet above ground, but in old plants the stem may rise for almost 20 feet before branching, and in these cases the axils are peculiarly inaccessible. Each of the short branches ends in a cluster of enormous leaves, forming a rosette very reminiscent of the terminal leaflets of a pineapple. The leaves, which are only 6 to 8 inches wide, may attain a length of 10 to 12 feet. They are stiff and strong, sword-shaped, and bordered by exceedingly sharp teeth along either margin. In addition, the midrib bears a row of keen spines on the underside. The close thickets formed by *Pandanus* are a formidable obstacle, particularly in view of the soft mud underfoot, the masses of saw-edged fallen leaves and the spiny nature of the prop roots. Progress necessitates a good deal of careful slashing, and even then the observer must expect to emerge with numerous cuts and deep scratches and with torn clothing.

Pandanus axils are very large and well formed, retaining considerable quantities of water, which is usually swarming with larvae. Fortunately this plant does not occur in areas favoured by *A. simpsoni* or it would inevitably become one of the most dangerous sources of this species. As most of the axils are high above ground, the best method of examination is for one person to hold the base of a leaf cluster steady while another lops off the soft branch. The entire cluster may then be removed from the thicket, the leaves cut short and the axils examined.

7. *Dracaena ugandensis.*

This shrubby *Dracaena*, locally known as " karamura," is much used for hedging cultivated plots, as noted by Gibbins (1942). It usually grows to a height of 12 to 15 feet (Pl. XIX, fig. 1). Several almost erect branches spring from the main stem near the ground, and these bear a profusion of rounded leaves almost throughout their length. The leaves are 4 to 5 inches long and about 2½ inches broad. At the tips of the branches the terminal leaves form deep cups, which retain water well. Apart from these cups the axils hold water in small quantities only, and this is very liable to spill when wind sways the slender branches. On the whole this plant must be regarded as a relatively poor breeding ground for mosquitoes.

8. *Dracaena steudneri* Schweinf. *ex* Engl.

This aborescent *Dracaena*, which grows to a height of 40 or 50 feet on favourable soil, is locally known as " kagorogoro." It is not cultivated, but is quite common along the edges of secondary forest, mainly in the river valleys, and isolated specimens occur among native crops. *D. steudneri* grows both in the lowlands and on the mountain slopes, and it is very common in the Toro highlands east of Bwamba. The many long branches terminate in rather palm-like rosettes of leaves, each leaf being about 3 feet long (Pl. XIX, fig. 2). The axils retain water well but seldom harbour larvae, a fact attributed by Gibbins to the exudation of toxic latex.

9. *Wild and garden cannas.*

In Bwamba a tall canna with small flowers of a brilliant red (*Canna orientalis* Rosc.) grows wild in the lowland areas (Pl. XX, fig. 1). It is sometimes also grown as borders and ornamental patches round native huts. Large-flowered garden hybrids have been introduced and are popular with the local Africans for ornamental purposes (Pl. XX, fig. 2). In some areas these have " escaped " and grow wild, the flowers becoming progressively smaller till they approximate in appearance to those of *C. orientalis*. The axils are capable of retaining water, but in quantities too small for mosquito development, except in the case of the cup formed by the terminal rolled leaf. All varieties are known locally as " maranga," and no attempt has been made to differentiate between them in mosquito work.

First Preliminary Survey.

PLANT AXILS EXAMINED.

The first work undertaken was a survey of mosquito breeding in plant axils in 6 mixed plantations around Bundibugyo, which lies in the middle of the inhabited area. In all, 600 plants were examined, 100 each of gonja, menvu, bitoke, colocasia (*Xanthosoma*), pineapple and *Dracaena ugandensis*. In view of the fact that the axils of a complete pineapple or *Dracaena* are very numerous indeed, single stems were chosen as the unit for these plants. The main results (Table I) were a confirmation of

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Note.—During early work the writer experienced much difficulty in distinguishing the various types of banana, etc., and in finding the local names. These often differ markedly from those given in the widely spoken Lutoro language of adjoining counties. A good example is that of the *Sansevieria* spp., which are known as " bugoigo " in Toro and " manusi " in Bwamba. For these reasons it has seemed appropriate to give rather full descriptions of the plants together with their local names, for the benefit of future workers. All the native names have been given in Lubwizi, the most widely spoken of the 5 Bwamba languages and one which is understood by almost all Africans in the county.

TABLE I.

Results of the preliminary survey of 6 plantations at Bundibugyo.

Species or type of plant		Gonja banana	Menvu banana	Bitoke banana	*Colocasia esculentum*	Pine-apple	*Dracaena ugandensis*
Plants	Plants examined	100	100	100	100	100	100
	Plants with water	95	68	48	97	91	94
	Plants with larvae	51	3	0	89	43	13
	Total axils	670	777	649	480	3,188	5,224
	Axils with water	237	97	53	215	517	1,014
	Per cent. axils with water	35	13	8	45	16	19
	Axils with larvae	65	3	0	135	60	13
	Per cent. axils with larvae	10	<1	0	28	2	<1
	Per cent. potential foci occupied	27	3	0	63	12	1
Mosquitoes	*Harpagomyia taeniarostris*	0	0	0	11	0	0
	Uranotaenia ornata	4	0	0	2	1	0
	Aëdes aegypti	4	3	0	4	0	0
	Aëdes simpsoni	90	1	0	317	102	1*
	Eretmapodites ferox	1	0	0	2	0	6
	Total larvae	99	4	0	336	103	7*
	Larvae per focus	1·5	1·3	0·0	2·5	1·7	?

Note that in the case of *D. ugandensis* the larval figures (marked with asterisks) are not correct, owing to predatory activity by *E. ferox*. This is explained in the text.

Gibbins' work, and showed that the important plants from the standpoint of mosquito breeding were colocasia, pineapple and gonja. Detailed comparison with Gibbins' figures was not possible, as in most cases he has not published the numbers of larvae taken in his surveys, restricting his figures to the numbers and percentages of plants and axils with larvae and the percentages of *A. simpsoni* among the total larvae.

When the data were reviewed in detail it was realised that the use of entire plants as units was far from satisfactory. For example, when colocasia and gonja were compared by entire plants the following figures were obtained :

	per cent.
Colocasias with larvae	89
Gonjas with larvae	51

These figures do not show adequately the great disparity between the plants : the colocasia sample yielded 317 larvae and the gonja sample only 90, in spite of the fact that the colocasia axils (480) were fewer than the gonja axils (670). Further, it was noted that while Gibbins used whole plants for survey purposes, in his tables he was compelled to discuss his results in terms of axils, to permit comparison between the various groups of plants. It was therefore decided that in later surveys axils and not whole plants would be the units employed. In considering the present sample the important figures seemed to be :

(*a*) The number of axils examined
(*b*) The number and percentage of axils with water
(*c*) The number and percentage of axils with larvae
(*d*) The percentage of water-containing axils which were inhabited.

The last figure (*d*) is an important one. It shows the percentage of available breeding places occupied and so gives an index of the preference of the mosquitoes for one plant as opposed to another—what might be termed the "breeding pressure." Applying these figures to the example quoted above, the following results were obtained :—

	Colocasia		Gonja	
	No.	Per cent.	No.	Per cent.
Axils examined	480		670	
Axils with water	215	45	237	35
Axils with larvae	135	28	65	10
Potential foci occupied ...		63		27

The degree of disparity between the two plants now becomes fully apparent for, though the gonja sample included more axils and more water-containing axils than the colocasia sample, the latter had both a larger number and a larger percentage of axils with larvae. Finally, in the case of the water-containing axils available for breeding, only 27 per cent. were occupied in the case of the gonja, as opposed to 63 per cent. in the case of the colocasia. The usefulness of these figures becomes still more apparent when a comparison between closely allied plants is desired, as in the case of the 3 groups of cultivated bananas.

Though the various figures discussed above help in assessing the comparative importance of the various plants in a given sample, there seems to be no method by which an accurate estimate can be made of the relative importance of the different plants in a stretch of country except in very obvious cases with gross differences (*e.g.*, gonja and bitoke bananas). Too many variables come into play—the relative abundance of the plants concerned, the number of axils per plant (which may vary from about 5 in colocasias to 2,000 or 3,000 in pineapples), the incidence of larvae in the axils and the mean number of larvae per axil, to mention only the most important. The best means at our disposal appears to be the comparison of equal numbers of axils from the various plants concerned, but it must be emphasised that at the best this gives only a very rough basis for comparison.

As the survey under consideration was planned essentially as a guide for future work, the size of the plants and their position in the plantations were recorded. The results of this subdivision need be discussed only in the case of the important plants (colocasia, pineapple and gonja). The samples were made up as follows :

	Colocasia	Pineapple	Gonja
Small plants near the edges ...	25	24	27
Small plants near the middle ...	25	26	25
Large plants near the edges ...	25	26	25
Large plants near the middle ...	25	24	23

The main findings were as follows :—

(*a*) *Colocasia.*

Though the percentage of axils containing water in small colocasias (45 per cent.) was about the same as that in large plants (44 per cent.), there was some difference between the percentage of potential foci occupied (58 per cent. in small and 67 per cent. in large plants). While this difference is probably not significant, it was decided that future work would be confined to large plants, as their axils are very easy to examine. When plants growing at the edges of mixed plantations (*i.e.* lightly shaded by the bananas) were compared with those growing in deeper shade in the middle, it was found that there was a marked disparity between the percentages of potential foci occupied (72 per cent. at the edges and 56 per cent. in the middle). The conclusion was, thus, that large plants growing round the edges of plantations were the most suitable for survey.

(*b*) *Pineapple.*

The percentage of axils containing water in small pineapple plants (16 per cent.) was the same as that in large plants (16 per cent.), and the percentage of potential foci inhabited was also very closely comparable (12 per cent. in small and 11 per cent. in large plants). This is obviously due to the fact that the leaves and axils, unlike those of colocasias, do not differ markedly in size in large and small plants, as the growth of the pineapple depends on the constant addition of new leaves at the end of the stem rather than on the growth of individual leaves. When plants at the edges of plantations were compared with those from the middle it was found that, as in the case of colocasias, those at the edges showed a much higher percentage of potential foci occupied (16 per cent.) than those in the middle (8 per cent.). Thus, in surveying mosquito breeding in the axils of pineapples, plants of any size at the edges of plantations are the most favourable.

(*c*) *Gonja.*

The percentage of water-containing axils in small gonja plants (45 per cent.) was considerably greater than that in large plants (29 per cent.) and in the small plants the percentage of potential foci occupied (29 per cent.) also compared favourably with that in large plants (25 per cent.). The study of full-grown gonjas is in any case rather difficult, necessitating the use of a long ladder (which must be self-supporting as the stems will not bear the weight). This leads to a greatly increased expenditure of time, and on a steep hillside the difficulties are further increased. When plants at the edges were compared with those in the middle, it was found that the percentage of potential foci inhabited was similar in both groups (25 per cent.) at the edges and 30 per cent. in the middle). In surveying gonjas, therefore, small plants (5 to 8 feet high) should be chosen, and their position in the plantation does not seem to be important.

A mixed plantation in Bwamba usually consists of bitoke, menvu and gonja bananas, in that order of prevalence, with an undergrowth of colocasias and some scattered pineapples. In addition, there is often a boundary hedge of *Dracaena ugandensis*. The above-mentioned findings show that a very fair estimate of the prevalence of axil-breeding mosquitoes can be made by surveying pineapples and large colocasias near the edges and small gonjas from any part of the plantation. Except for detailed work the other plants may be ignored.

Mosquito Larvae Collected.

Larvae totalling 549 specimens, belonging to 5 species, were taken in the preliminary survey :

(*a*) *Harpagomyia taeniarostris*, Theo.

Eleven larvae of this species were obtained, all from colocasia axils; they represented 2 per cent. of the total larvae. This highly specialised mosquito is of no practical importance, as it does not suck blood.

(*b*) *Uranotaenia ornata* var. *musarum*, Edw.

Seven larvae of this species, representing 1 per cent. of the total, were taken from gonja, colocasia and pineapple. This species is not known to bite man.

(*c*) *Aëdes* (*Stegomyia*) *aegypti*, L.

Records of this "domestic" species breeding in plant axils are of considerable interest. Its unusual habits in Bwamba (where it often breeds in tree-holes, sometimes in uninhabited forest) have been discussed in a previous communication (Haddow, 1945 a). Larvae were taken in the present survey from axils of gonja, menvu and colocasia, the total of 11 representing 2 per cent. of the entire sample. As the larvae of *A. aegypti* are very similar to those of *A. simpsoni*, it may be mentioned that in this preliminary survey all larvae of these species were reared separately in tubes lightly plugged with cotton wool in order to obtain adults to confirm the identifications.

(*d*) *Aëdes* (*S.*) *simpsoni*, Theo.

The survey showed clearly the very marked preponderance of this important species among the axil-breeders of Bwamba plantations. The catch of 511 larvae represented 93 per cent. of the total for all species, and larvae were taken from all the plants studied except bitoke—from which no larvae of any kind were obtained.

(*e*) *Eretmapodites ferox*, Haddow

This species, so far known only from Bwamba, has been described in a previous communication (Haddow, 1946). It closely resembles *E. dracaenae*, Edw., but can be distinguished by differences in the male terminalia. The larva is actively predatory on other mosquito larvae, and will devour large numbers of *A. simpsoni* in a short time. Nine larvae, representing about 2 per cent. of the total for all species, were taken.*

Treatment and Identification of Larvae.

With regard to the treatment and identification of larvae, it was found essential to deal with samples immediately. The larvae of all the local *Eretmapodites* spp. are predatory to a greater or lesser degree, and several species are quite common in axils. Any of these easily recognised larvae seen in the field should be picked out and taken to the laboratory in a separate container. The sample of larvae from *Dracaena ugandensis* in the present survey is an example of what may occur if this precaution is neglected. It will be noted (Table I) that the number of larvae recorded (7) is less than the number of inhabited axils. Actually, about 20 larvae were taken, and these are all thought to have been *A. simpsoni* with the exception of the 6 *E. ferox*. By the time that the sample had reached the laboratory—about 2 hours later—only one of the *Aëdes* larvae was left.

In examining a sample it is considered important that larvae in all stages, and not merely 4th-stage larvae, should be identified. With a little practice this should give no difficulty in the case of a restricted group like the axil-breeders. Among the species encountered in Bwamba, the only 2 which are liable to be confused in the early stages are *A. simpsoni* and *A. aegypti*, and even here careful examination will

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**Note.*—At this point it is convenient to comment on Gibbins' determinations of *Eretmapodites* spp. from Bwamba. He records *E. dracaenae*, *E. leucopus*, *E. chrysogaster* and *E. quinquevittatus*. His African assistant informed the writer that all of these were identified as larvae, except *E. quinquevittatus*, in which case the record was based on pupae. It is obvious that the records of *E. dracaenae* refer to the new species *E. ferox*, which is abundant in Bwamba and cannot be reliably distinguished in the larval state. *E. dracaenae* has not been found in Bwamba by the writer in the course of more than 4 years' work. The records of *E. leucopus* should be referred to *E. leucopus* subsp. *productus*. Typical *E. leucopus* is not known to occur anywhere near Bwamba. Study of the larvae of the *E. chrysogaster* group has shown that the characters formerly used in distinguishing the larvae of *E. chrysogaster* and *E. semisimplicipes* are not reliable. Both species are common in plant axils in Bwamba, and it is likely that other species of the group may also frequent this environment. Gibbins' record should thus be referred to the " *E. chrysogaster* group ". The examination of several thousand *Eretmapodites* species from Bwamba has failed to reveal *E. quinquevittatus*, and indeed all Uganda records of this species must be regarded as very doubtful. At the time of Gibbins' survey the pupa of *E. inornatus* had not been described, but the writer's observations (1946) have shown that it has a remarkable resemblance to that of *E. quinquevittatus* (this rather extraordinary pupa had previously been considered unique). *E. inornatus* larvae, moreover, have been taken by the writer from plant-axils in the area from which Gibbins recorded *E. quinquevittatus* (Bundibugyo-Bumadu footpath). There seems to be little doubt, therefore, that Gibbins' record should be referred to *E. inornatus*.

reveal many points of difference. As, however, the distinction of these species is a matter of importance, the following procedure was used: If a sample was seen to contain larvae which seemed to be *A. aegypti*, the larger specimens were picked out and reared separately; so that every record of this species in the present communication is supported by the breeding-out of at least 1 adult. In the course of plant-axil work *A. aegypti* occurred once in pineapples and 3 times in colocasias (during a monthly survey of *X. sagittifolium*) apart from those taken in the samples discussed above. It was also taken once in a plant axil in a large survey of roadside and forest-edge breeding and several times in large random samples taken from axils to provide material for a study of *Eretmapodites* larvae.

Pupae taken in plant axils were considered as part of the sample. They were usually bred out, but any which died were identified in the pupal state.

Second Preliminary Survey.

At the conclusion of the first survey another preliminary study was made, to gauge the incidence of larvae in different types of lowland country. This second survey included the collection of adult mosquitoes and of larvae from plant axils, tree-holes, leaf pools and ground pools, though only the axil work need be considered here. The survey covered 3 lines:

(*a*) Five miles of the main road, from Bundibugyo to Bubukwanga. Open country with only 1 very small strip of forest. Much cultivation. Total plants examined—150.

(*b*) Three miles of native footpath, from Hakitengya to Buhanda. Mixed country with open ridges and 2 deep river valleys filled with relict primary forest. Much cultivation. Total plants examined—150.

(*c*) Five miles of native footpath, from Buhanda to Bubukwanga, along the edge of the Semliki Forest. Cultivated country and bush bordering on virgin rain forest. Total plants examined—200.

These 3 surveys, embracing open cultivated country, mixed cultivation and forest and forest-edge country, respectively, included samples from each plantation along the chosen lines, which formed a rough triangle. The samples included equal numbers of gonja and colocasia plants in each case, but the results may be considered collectively here. It will be seen (Table II) that in all 3 types of country breeding pressure was high, the mean percentage of available foci occupied being 53, and that *A. simpsoni*

TABLE II.

Results of a survey of gonja and colocasia plants in 3 types of lowland country.

Survey	(*a*) Open cultivated country	(*b*) Mixed forest and cultivation	(*c*) Forest-edge country	Totals
Total axils	766	737	925	2,428
Axils with water	516	460	498	1,474
Axils with larvae	335	232	209	776
Per cent. potential foci occupied ...	65	50	42	53
H. taeniarostris	98	123	112	333
U. ornata v. *musarum*	36	3	4	43
A. (*S.*) *aegypti*	—	2	—	2
A. (*S.*) *simpsoni*	725	184	132	1,041
E. chrysogaster group	3	—	71	74
E. ferox	41	46	7	94
E. leucopus subsp. *productus*	25	—	3	28
All larvae	928	358	329	1,615

Equal numbers of these plants were examined, though the results are given collectively. The numbers of plants examined were: Survey a, 150; Survey b, 150; Survey c, 200.

was everywhere the commonest larva, 1,041 being taken. This represents 64 per cent. of the total for all species (1,615).

These preliminary surveys having established the importance of colocasia, gonja and pineapple from the standpoint of mosquito breeding and having shown that *A. simpsoni* was widespread in the lowland country, more detailed work was begun. It had been concluded that the water content of the axils was likely to be the main factor influencing the preference of mosquitoes for one plant over another, and the next survey undertaken (which covered almost all the plants likely to harbour larvae) included measurements of the amounts of water retained by the axils.

Observations on the Water Content of Plant Axils.

The first intention was that water measurements in plant axils should be combined with a survey of the larvae contained, but this was found impracticable for various reasons. The most important of these was that in order to withdraw all the water from an axil a very fine pipette must be used. This is of course unsuitable for the collection of larvae, as it will not draw up large specimens and is very liable to damage small ones. In the case of larval surveys it has been found best to use a fairly coarse pipette and to wash out the axil with several changes of clean water.

The plan finally employed was an individual measurement of the content of 1,000 water-bearing axils of each type of plant. This allowed a normal to be established, which could be applied to samples in which the collection of larvae was the sole object. The results are listed in Table III, in which also various forms of average are given in the case of plants which harboured *A. simpsoni* (with the exception of *D. steudneri*). The implications will be discussed in the next section.

TABLE III.

Results of measurements of the water-content of plant axils.

Plant	Total content of 1,000 axils	Largest content of any single axil	Arith-metic mean	Geo-metric mean	Median	Mode
Wild banana	166,920	1,754·8	166·9	—	—	—
C. esculentum	14,563	97·6	14·6	—	—	—
P. chiliocarpus	9,258	97·4	9·3	—	—	—
X. sagittifolium... ...	6,767	26·2	6·8	5·8	6·2	5·0
D. steudneri	4,620	32·5	4·6	—	—	—
Gonja banana	4,511	19·4	4·5	3·8	3·6	1·8
Pineapple	4,181	11·6	4·2	3·7	3·8	3·0
D. ugandensis	3,516	16·6	3·5	2·8	2·8	1·4
Bitoke banana ...	3,113	15·4	3·1	2·8	2·9	2·2
Menvu banana	2,906	18·6	2·9	2·6	2·9	2·9
Cannas	2,777	15·8	2·8	2·4	2·5	2·0
Sansevieria spp. ...	1,487	7·2	1·5	1·3	1·4	1·2

In the case of each plant the contents of 1,000 water-bearing axils were measured. All amounts are in cc. The arithmetic mean content is given for each kind of plant, and in the case of plants harbouring *A. simpsoni* (except *D.steudneri*) various other forms of average are also given.

The measurements concerned were all made in wet weather when the axils were full, and in various cases several weeks of waiting were necessary before conditions became favourable. It was only at the conclusion of the survey that the writer realised that a much simpler and perhaps preferable method could have been used, namely, to fill the axils with water and then measure the contents. While the measurements recorded here are based on natural conditions, the filling method, though artificial, would give a better guarantee that the axils were full to capacity. In the present samples, to avoid selection, the contents of every water-bearing axil of a single plant was measured before moving to another.

Buxton and Hopkins (1927) working on an axil-breeding mosquito in Samoa were led to wonder whether the fluid in the axils of the taro (*C. esculentum*) could be secreted by the plant in whole or in part, as they noted that all rainfall on the lamina drains away by the tip of the leaf and cannot enter the axils. An experiment in Bwamba, however, indicates that the fluid is derived from direct rainfall. Two small *Xanthosoma* plants ("binongwe byekijungu" variety) were established in separate boxes of earth. All the water was then withdrawn from the axils with a fine pipette. One plant was placed under a sheltered verandah and the other in the open. The axils of the plant on the verandah remained dry while those of the other filled to capacity with the first shower and thereafter retained a normal content. Except for the leaf-outline, *Xanthosoma* closely resembles *C. esculentum* in structure.

Buxton and Hopkins (1927) found traces of chloride in the axils, the fluid giving a slight precipitate with silver nitrate. They suggest that this may have been due to the proximity of the sea, traces of wind-borne spray being gradually concentrated in the axils. This seems to be the explanation, as in Bwamba the fluid from *Xanthosoma* axils does not give a precipitate with silver nitrate. It is, indeed, difficult to conjecture from what source the larva obtains its supplies of chloride. One possibility is that enough may be stored in the egg to carry the larva through to adult life.

Survey of Twelve Types of Plant.

The results of this survey are shown in Table IV. The columns, reading from left to right, are arranged so that the percentages of potential foci occupied are in descending order. It will be noted that 3 plants (wild banana from the mountain slopes, *Pandanus* from the Semliki Forest and *C. esculentum* from the mountain) yielded no *A. simpsoni*. In view of the fact that these plants harboured huge numbers of other larvae, this is thought to be merely a reflection of the fact that they grow in environments apparently unsuited to this species. All the other plants yielded *A. simpsoni*, the most important being colocasia (*X. sagittifolium*), gonja and pineapple, in that order. The remainder—*Sansevieria* spp., *D. ugandensis*, menvu, cannas, *D. steudneri* and bitoke—contained far fewer larvae. There was a pronounced gap between pineapple, with 935 larvae (902 *A. simpsoni*) and *Sansevieria*, the next most favourable plant, with only 218 larvae (all *A. simpsoni*). The reasons for this will be discussed further below.

It should be noted that in the favourable types of plant listed on the left of the table the variety of species is great, whereas in those to the right the small yield of larvae is almost or entirely composed of *A. simpsoni*. This is probably a reflection not only of the general abundance but also of the hardiness of the *A. simpsoni* larva, which is easy to rear in the laboratory in all sorts of water and which is very much less delicate than the more highly specialised axil-breeders such as *H. taeniarostris*. It should be remembered that in other parts of Africa *A. simpsoni* is common in tree-holes and that it has been recorded from tins, bottles, coconut shells and tubs (Hopkins, 1936). It may further be mentioned that the larvae found in the axils of unfavourable plants are almost invariably in the first or second instar, and it seems very likely that many of them must fail to complete their development. The abundance of *Culex nebulosus* larvae in axils has a certain interest. In Bwamba this species seems almost ubiquitous, and its larvae have been taken in almost every type of breeding water.

In Table IV, as mentioned above, the plants are listed from left to right so that the percentages of potential foci occupied are in decreasing order of magnitude. It will be noted that there is an exceedingly close correspondence between this percentage and the numbers of larvae taken and that this is closer than the correspondence between the numbers of larvae and the crude numbers of axils

TABLE IV.

Results of a survey of 12 kinds of plants.

	Type of Plant	Wild banana	*Pandanus chiliocarpus*	*Xanthosoma sagittifolium*	Gonja banana	Pineapple	*Colocasia esculentum*	*Sansevieria* spp.	*Dracaena ugandensis*	Menvu banana	Cannas	*Dracaena steudneri*	Bitoke banana	Totals and general rates
Plant data	Axils with water	732	611	418	633	651	471	432	548	472	245	229	112	5,554
	Axils with larvae	602	420	264	369	316	111	108	74	6	4	5	1	2,280
	Per cent. potential foci occupied ...	82	69	63	58	49	24	25	14	<2	<2	2	<1	41
Mosquito data	*H. taeniarostris*	—	18	56	2	—	12	—	—	—	—	—	—	88
	U. ornata v. *musarum*	26	1,647	324	14	25	7	—	—	—	—	—	—	2,043
	Uranotaenia sp. incog.	—	128	—	—	—	—	—	—	—	—	—	—	128
	A. (*S.*) *aegypti*	—	—	—	—	6	—	—	—	—	—	—	—	6
	A. (*S.*) *simpsoni*	—	—	1,321	1,203	902	—	218	126	32	10	8	1	3,821
	E. chrysogaster group	—	3	18	—	—	—	—	—	—	—	—	—	21
	E. ferox	—	—	5	2	—	—	—	24	—	—	—	—	31
	E. leucopus subsp. *productus*	—	—	6	—	1	—	—	—	—	—	—	—	7
	C. (*C.*) *nebulosus*	218	20	45	—	1	—	—	—	—	—	—	—	284
	Culex sp. near *trifilatus*	7	—	—	—	—	—	—	—	—	—	—	—	7
	Culex sp. near *toroensis*	11	—	—	—	—	—	—	—	—	—	—	—	11
	C. (*C.*) *musarum*	9,235	—	—	—	—	805	—	—	—	—	—	—	10,040
	Total larvae	9,497	1,816	1,775	1,221	935	824	218	150	32	10	8	1	24,321
	Approximate number of larvae per inhabited axil	16	4	7	3	3	7	2	2	5	3	2	1	11

One thousand axils were studied in each type.

inhabited. Actually, it has been found that in the case of plants harbouring *A. simpsoni* there is a highly significant positive correlation between the numbers of this species taken and the percentage of potential foci occupied, r being +0·86 and t 4·13 (in this instance a value of 3·71 for t would imply that an r of this magnitude would be unlikely to occur in uncorrelated series more often than once in 100 trials).

Turning to the relationship between the amounts of water retained by the axils of different plants and the numbers of larvae taken, it has seemed best to restrict the analysis to the single species *A. simpsoni.* This eliminates the wild banana, *Pandanus* and *C. esculentum* from the discussion, and it has been further decided that *D. steudneri* should also be omitted as in its case a quite unrelated factor—the exudation of toxic latex—comes into play. In the case of the other plants the various averages for water-content listed in Table III were applied to the samples under consideration, this allowing an expected water content for each sample to be worked out. Thus, in the case of *Xanthosoma*, for example, the sample showed 418 axils with water. This figure was multiplied in turn by the arithmetic mean, geometric mean, median and modal water content for *Xanthosoma* axils, as determined by the water content survey (*i.e.* 6·8, 5·8, 6·2 and 5·0 cc. respectively), and the resulting figures of 2,842, 2,424, 2,902 and 2,090 were regarded as various expressions of the expected water content of the thousand-axil sample. This procedure was applied similarly to the other plants concerned.

Where the arithmetic mean was used to calculate this expected water content, it was found that there was significant correlation between the amounts of water in the axils of the various plants and the numbers of *A. simpsoni* taken, r being +0·74 and t 2·81 (where the borderline significance of t—representing 1 chance in 20—is 2·45 and the high-significance value—representing 1 chance in 100—is 3·71). In the case of the geometric mean r was +0·79 and t 3·17, the median gave r=+0·78 and t=3·03, and the mode gave r=+0·74 and t=2·81. Thus in the case of each of the four types of average, it was found that there was a significant degree of positive correlation between the numbers of *A. simpsoni* taken in the various plants and the expected water content of the particular plant samples concerned. It will be seen that the closest correlations were obtained where the geometric mean and the median were employed, the arithmetic mean and the mode being slightly less satisfactory. As, however, the differences are not great in any case, it is thought that the use of the arithmetic mean—which is by far the simplest to compute—is probably adequate for most ordinary purposes.

Though this observation shows that the numbers of *A. simpsoni* tend to increase with increasing capacity of the axil, it is obvious that there must be a limiting factor, *i.e.* when the axil exceeds a certain size or capacity it must cease to provide the conditions favoured by axil-breeders, becoming what may be described as a miniature pool. In this connection it is interesting to recall experiments carried out in the South Seas by Buxton and Hopkins (1927) which showed that the " small-container breeders," *Aëdes variegatus* and *A. argenteus*, seemed to prefer medium-sized vessels for oviposition when provided with dishes of 3 sizes (10, 15 and 30 cm. diameter). These authors found that during 9 weeks 145 eggs (10 layings) were deposited in the 10 cm. dish, 318 eggs (26 layings) in the 15 cm. dish and 86 eggs (6 layings) in the 30 cm. dish. The present investigation shows a similar type of behaviour among axil-breeders. The plants were divided into 4 groups, according to mean axil capacity, as follows : with less than 4 cc., with 4 to 8 cc., with 8 to 16 cc. and with over 16 cc. The total expected (arithmetic) mean water content for the group was then divided by the total larvae yielded by the group, the resulting figure representing the population density or mean water volume per larva. The results were as follows :—

Group 1. *Small axil content, under* 4 *cc.*

The plants in this group are bitoke, *Sansevieria*, cannas, menvu and *D. ugandensis.* The rate is approximately 1 larva for each 12 cc. of water.

Group 2. *Medium axil content,* 4 *to* 8 *cc.*

The plants in this group are pineapple, *Xanthosoma* and gonja. The rate is approximately 1 larva for each 2 cc. of water.

Group 3. *Large axil content,* 8 *to* 16 *cc.*

The plants in this group are *Pandanus* and *C. esculentum.* The rate is approximately 1 larva for each 5 cc. of water.

Group 4. *Very large axil content, over* 16 *cc.*

The only plant in this group is the wild banana, as *D. steudneri* is again omitted from consideration. The rate is approximately 1 larva for each 13 cc. of water.

It is of course obvious that water content is only one of many factors influencing the preference of the mosquitoes for one plant over another. In the first group, for example, bitoke bananas, with a mean water content of 3·1 cc. per axil, yielded only 1 larva ; while *Sansevieria*, with a mean content of only 1·5 cc. per axil, yielded 218. Of course the *Sansevieria* produced a much larger number of water-retaining axils than did the bitoke (432 and 112 respectively with expected volume of 648 and 347 cc.) ; but this is by no means a full explanation, as the bitoke yielded only 1 larva from 347 cc. while *Sansevieria* yielded approximately 1 larva for each 3 cc. The general principle, however, seems fairly clear, and it is interesting to note that while the wild banana produced immense numbers of larvae, their population density (1 per 13 cc.) was lower than that in the unfavourable plants of Group 1 (1 per 12 cc.). Nothing can be deduced at the moment about *D. steudneri*, as the present study contains no observations on oviposition. This plant is obviously very unfavourable for larval development, and it may be noted that, of the 8 larvae taken, 7 were very small and the eighth, though it had reached the third instar, was moribund. This does not necessarily imply that females rarely oviposit in the axils of this species, as it is known that female *Aëdes* will lay in waters containing poisons which are rapidly fatal to the hatching larvae (Buxton and Hopkins, 1925, 1927).

The general conclusion is thus that the capacity of the axils of a given plant species is likely to have a pronounced effect on the numbers of larvae found therein—provided the fluid is not toxic—and that in Bwamba the optimum seems to lie between 4 and 8 cc.

A Monthly Survey of *Xanthosoma sagittifolium.*

A survey of lowland colocasias of the "binongwe byekijungu" type (the green-stemmed variety of *X. sagittifolium*) was carried out monthly at Bundibugyo from March 1942 until February 1943. The plants studied were in the 3 best plantations of the 6 included in the preliminary axil survey.

Unfortunately this survey had been running for 3 months before it was realised that the axil and not the entire plant was the best unit for work of this type. It was decided that the sample first employed—100 plants per month—must be continued ; but as a result, different numbers of axils were examined in different months. In Table V, however, where the results of the survey are shown, additional standardised figures are given, listing the expected yield of *A. simpsoni* and of all larvae per thousand axils per month.

Little need be said of the species taken, except to note that *H. taeniarostris*, *U. ornata* var. *musarum* and *A. simpsoni* were the only larvae present in every month of the survey, and that they represented 2, 15 and 72 per cent. respectively, of the total for all species. All the other species except *A. aegypti*, however, were very regularly present, though their numbers were never great.

With regard to the monthly fluctuations in the numbers of larvae taken, it must be emphasised that local rainfall had little influence. This is obviously due to the

TABLE V.

Results of a monthly survey of *Xanthosoma sagittifolium* around Bundibugyo, lasting for 1 year.

Month	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Totals	Per cent.
Total axils	480	552	573	585	536	621	598	544	507	484	338	356	6,174	—
Axils with water	215	371	184	330	243	426	335	290	305	335	217	32	3,283	53
Axils with larvae	135	200	121	193	161	270	155	125	203	115	20	9	1,707	28
Per cent. potential foci occupied ...	63	54	66	59	66	63	46	43	67	34	9	28	—	52
H. taeniarostris	11	1	1	16	8	30	2	18	36	12	1	6	142	2
U. ornata v. *musarum*	2	136	5	79	65	155	63	89	320	158	6	4	1,082	15
A. (*A.*) *aegypti*	4	3	3	—	—	—	—	—	—	—	—	—	10	<1
A. (*A.*) *simpsoni*	317	117	230	784	387	538	267	615	1,058	746	74	55	5,188	72
E. chrysogaster group	—	3	8	—	26	42	6	24	18	93	70	—	290	4
E. ferox	2	11	13	13	19	2	16	10	5	2	—	—	93	1
E. leucopus subsp. *productus*	—	1	7	2	12	7	—	7	6	—	6	—	48	<1
C. (*C.*) *nebulosus*	—	—	—	9	9	11	—	37	45	253	—	—	364	>4
Total larvae	**336**	**272**	**267**	**903**	**526**	**785**	**354**	**800**	**1,488**	**1,264**	**157**	**65**	**7,217**	—
Expected *A. simpsoni* per thousand axils	660	212	401	1,340	722	866	446	1,131	2,087	1,541	219	155	—	—
Expected total larvae per thousand axils	700	493	466	1,544	981	1,264	592	1,471	2,935	2,612	464	182	—	—

One hundred plants examined per month.

fact that a light shower will fill the axils to capacity and that heavy rain cannot fill them further. In addition to this it should be noted that the axils, once filled, retain water for a considerable period and thus 3 to 4 light showers per month are, under ordinary circumstances, sufficient to permit normal larval populations to be maintained. The only way in which local rainfall has an influence on larval populations quantitatively is indirectly, through the growth of the plants.

The fluctuations in larval numbers from month to month are obviously dependent on the growth and harvesting of the colocasias. In this connection it must be emphasised that the present survey is a particular example, applicable only to the period during which it was carried out, as the planting and cropping of colocasias does not occur in the same months each year. In considering the present samples the expected figures for *A. simpsoni* and total larvae have been employed, though it so happens that in this particular example they follow exactly the same trends as the crude figures. The results are analysed below, month by month :

March 1942

Plantations containing scattered residual plants from the last harvesting period (1941) and a small number of newly planted colocasias. Harbourage poor. Total larvae (700) and *A. simpsoni* (660) few.

April 1942

The last old plants now removed and planting continued in heavy rain. Harbourage now very poor as almost all plants are very small. Total larvae (493) and *A. simpsoni* (212) very few.

May 1942

Intensive planting continued in heavy rain. The April plants not yet full grown, so harbourage still rather poor. Total larvae (466) and *A. simpsoni* (401) still few.

June 1942

Plantations now dense and March–April plants approaching full growth. Harbourage excellent. Total larvae (1,544) and *A. simpsoni* (1,340) abundant.

July 1942

Harvesting of early (March–April) plants. These replaced by new plantings in readiness for the next heavy rain. Late (May) plants now approaching full growth, but plantations greatly thinned out by the harvesting of the first crop and harbourage greatly diminished. Total larvae (981) and *A. simpsoni* (722) substantially reduced.

August 1942

Plantations now fairly dense, but most late (May) plants now harvested and July plants still rather small. Harbourage quite good. Total larvae (1,264) and *A. simpsoni* (866) numerous.

September 1942

The last May plants now harvested and July–August plantings still small. Plantations rather open and harbourage poor. Total larvae (592) and *A. simpsoni* (446) few.

October 1942

July–August plants now large and plantations very dense with excellent harbourage. Total larvae (1,471) and *A. simpsoni* (1,131) numerous.

November 1942

Plantations now very dense and harbourage very good. A little harvesting of the third crop begun. Total larvae (2,935) and *A. simpsoni* (2,087) very numerous.

December 1942

Harvesting of the third crop. Before this shelter was excellent. Little planting done as the expected dry weather of January–February not suitable for the establishment of young plants. Sample (taken before harvesting) showed total larvae (2,612) and *A. simpsoni* (1,541) to be very numerous.

January 1943

No activity in the plantations. Scattered residual plants and a few young plants only. Harbourage very poor. Total larvae (464) and *A. simpsoni* (219) scarce.

February 1943

Still very little activity in the plantations. No large plants left. Harbourage very poor. Total larvae (182) and *A. simpsoni* (155) very scarce.

This shows that to gain a true idea of the status of *A. simpsoni* in a given locality a sample taken all at one time is not adequate, and that repeated collections should be made at different stages of plant growth. During the year concerned, the axils only once dried out completely (for a very short period at the beginning of February) but the larval populations had been re-established within 10 days of the first light shower. *A. simpsoni* is of course well adapted to survive dry weather, as the eggs are drought resistant. It should further be remembered that at a time when this species is scarce in colocasias it may be (and was) quite abundant in gonjas, which are not harvested in the same way as colocasias. In the case of gonjas it is usual to plant out suckers in a fruiting plot, and the old plants—which give useful shade—are not cut down for mulching until the young plants are well established, so that they often remain standing long after their fruit has been harvested. These notes refer to the Bwamba lowlands and are not necessarily applicable elsewhere.

Observations on Scrapings from Dry Axils.

Dunn (1926) has shown that if scrapings from dry tree-holes are placed in water, the larvae of various species will hatch from drought-resistant eggs. During the dry period at the end of January and the beginning of February 1943 this method was applied to colocasias (*X. sagittifolium*).

The first collection was made from 100 axils. Owing to a misunderstanding, the collector brought in damp débris from axils which were not quite dry. This damp material contained numerous small living larvae of mosquitoes and Psychodids and was discarded.

A second collection was then made from 1,000 dry axils. In each case the loose débris was first collected and the axil was then washed out with several changes of water, the interior also being scraped with a small spoon. Even in this dry material 4 living larvae were found. These were present in the minute film of water between the stem and petiole at the very bottom of the axil. Buxton and Hopkins (1927) give some interesting notes on the unusual powers of wandering on damp surfaces possessed by the larva of the axil-breeder, *Aëdes kochi* var. *samoana*, a characteristic which must be of great value to the larva in times of drought. It is not known whether any of the Bwamba species has similar powers, but the present observations show that they can resist dry conditions to a rather remarkable degree. The four larvae were discarded and the remaining material was placed in bowls of water in mosquito-proof cages.

Larvae appeared on the second and third days, the total being 31 (24 *A. simpsoni* and 7 *H. taeniarostris*). After a week, as no further hatchings had taken place, the material was discarded. On the whole, the yield of larvae was smaller than had been anticipated. *A. simpsoni* is known to have drought-resistant eggs, and the present experiment shows that they may lie in dry axils waiting for rain exactly as in the case of the tree-hole breeders (including *A. simpsoni*) studied by Dunn. *H. taeniarostris* on the other hand, has not been recorded as possessing drought-resistant eggs.

Temperature Measurements in a *Xanthosoma* Axil.

Teesdale (1941), working at Mombasa, noted that the period covered by larval development in axils may be very prolonged. In his experiments he enclosed plants known to contain larvae in mosquito-proof cages and thereafter noted the dates of emergence of the adults. In the case of 2 of the banana plants, adults continued to emerge till the 19th day after the enclosure of the plant and in 1 of the pineapples adults continued to emerge for 29 days. Buxton and Hopkins (1927) have shown that the temperature of water in colocasia axils is slightly lower than that of the air. In 10 axils they found the temperature to vary from 25·5 to 29·0°C. (mean 27·5°C.) at a time when the air temperature was 30·5°C. They further noted that relatively slight temperature differences had a significant effect on the duration of the larval and pupal stages of the " small-container " breeder, *Aëdes argenteus*.

It therefore seemed possible that the slow development noted by Teesdale might be due to low temperature in axils, particularly as the species with which he was mainly concerned (*A. simpsoni*, *A. aegypti* and *A. metallicus*) all develop very rapidly under laboratory conditions. The following experiment was therefore carried out to compare the temperature in a colocasia axil with that of the surrounding air.

A maximum and a minimum thermometer were placed in a large water-containing *Xanthosoma* axil in the middle of a plot. Another pair of thermometers were placed in a Stevenson screen about 6 feet away. Conditions were not ideal, as thermometers in air or water should have a clear space around the bulbs, and in this experiment, though the axil was a large one, it was impossible to avoid contact between the bulbs and the sides of the axil. Further, a Stevenson screen should be erected in an open space, whereas in this instance it was necessary to place it actually among the colocasias so that a close comparison with the axil might be obtained. Readings were continued for a week. The results are shown in Table VI.

TABLE VI.

Temperature readings on 7 successive days in a *Xanthosoma* axil and in an adjoining Stevenson screen.

Day	1	2	3	4	5	6	7
Minima—							
Axil	17·0	18·0	18·0	18·0	18·0	17·0	18·0
Screen	17·0	18·0	19·0	19·0	18·0	17·0	18·0
Maxima—							
Axil	23·0	25·0	25·0	25·0	24·0	25·0	26·0
Screen	24·0	28·0	28·0	27·0	27·0	27·0	28·0
Means—							
Axil	20·0	21·5	21·5	21·5	21·0	21·0	22·0
Screen	20·5	23·0	24·5	23·0	22·5	22·0	23·0
Ranges—							
Axil	6·0	7·0	7·0	7·0	6·0	8·0	8·0
Screen	7·0	10·0	9·0	8·0	9·0	10·0	10·0

All readings are in °C. Owing to the small size of the thermometers it was thought best to read to the nearest whole degree.

It will be seen that the minima corresponded rather closely, sometimes the axil and the screen showing the same temperature and sometimes the axil being slightly cooler than the screen. In the case of maxima, however, the screen consistently showed a higher temperature than the axil. The greatest observed difference between minima was 1°C., between maxima 3°C., between means 2·5°C. and between ranges 3°C.

A Stevenson screen is a well-insulated structure, yet the temperature in a colocasia axil seems to be still more equable, as is shown by a comparison between the absolute and mean minima, maxima and ranges :

	Abs. Min.	Abs. Max.	Abs. Range	Mean Min.	Mean Max.	Mean Range
Axil	17	26	9	17·5	24·5	7·0
Screen	17	28	11	18·0	27·0	9·0

It thus becomes apparent that larvae developing in a colocasia axil experience a remarkably stable temperature and that this temperature tends to be lower than that of the surrounding air. It is also thought likely that the air temperature in a large *Xanthosoma* plot is likely to be lower than that of more exposed situations.

Observations on the Mosquito Output of *X. sagittifolium.*

The preceding sections have dealt with the numbers of larvae found in samples taken from plant axils, but these do not necessarily give a true picture of the mosquito output of the plants concerned. This applies particularly to vulnerable larvae such as that of *A. simpsoni*, for the presence of a single *Eretmapodites* larva in an axil will ensure that few if any of the other larvae in that axil will complete their development. The following example may serve to show how marked is the influence of predators on the populations of other mosquito larvae in axils.

At the time when the thousand-axil sample from *Xanthosoma* was being collected at Bundibugyo, larvae of *E. chrysogaster*, *E. ferox* and *E. leucopus* subsp. *productus* were being gathered for systematic work at Bubukwanga, some 5 miles away, where they were particularly abundant. To gauge the effects of their predatory activity on the other species, a thousand-axil sample was collected at Bubukwanga, for direct comparison with the Bundibugyo material. The results (Table VII) show that though

TABLE VII.

Samples from *Xanthosoma* axils.

Locality	Bubukwanga	Bundibugyo
Axils with water	663	418
Axils with larvae	443	264
Per cent. potential foci occupied ...	67	63
H. taeniarostris	504	56
U. ornata v. *musarum*	14	324
A. (*S.*) *simpsoni*	615	1,321
E. chrysogaster group	67	18
E. ferox	87	5
E. leucopus subsp. *productus* ...	54	6
C. (*C.*) *nebulosus*	—	45
Total larvae	**1,344**	**1,775**
Approximate number of larvae per inhabited axil	3	7

Samples taken simultaneously in 2 localities, 1,000 axils each, to show the influence of predators (*Eretmapodites* spp.) on the populations of other species.

the Bubukwanga sample included more axils with water and more axils with larvae than did that from Bundibugyo, the yield of larvae was much smaller, particularly in the case of *A. simpsoni* and *U. ornata* var. *musarum*. At Bundibugyo the number of larvae per inhabited axil was about 7, while at Bubukwanga it was only 3. This difference was almost certainly due to the relative abundance of *Eretmapodites* larvae at Bubukwanga. At Bundibugyo 29 of these were taken, representing only about 2 per cent. of the total larvae, while at Bubukwanga the yield was 208, representing 15 per cent. of the total. It has been noted elsewhere (Haddow, 1946) that as a rule *Eretmapodites* larvae occur singly in axils, obviously on account of their voracious nature. It would therefore be reasonable to assume that in the Bubukwanga sample the 208 *Eretmapodites* larvae dominated about 200 (30 per cent.) of the 663 water-containing axils, while at Bundibugyo they probably dominated only about 25 (6 per cent.) of the 418 available axils. Shannon (1931) has pointed out that the restless movements of *Stegomyia* larvae render them peculiarly liable to attack by predators, and this obviously applies to *A. simpsoni*. The same may be held true of *Uranotaenia* larvae which also have restless habits. With regard to *H. taeniarostris*, which was particularly abundant at Bubukwanga, it should be noted that the larva spends much of its time simply lying on the bottom and making only short excursions to the surface. It is thus rather well adapted to escape the attentions of the predatory *Eretmapodites*, which seldom attack a stationary larva.

Another factor which tends to reduce the output of adults is excessive growth of Protozoa on the larvae. Most plant-axil larvae will be found to bear a few stalked ciliates of the *Vorticella* and *Carchesium* types. Under certain conditions—probably the presence of a large food-supply or particularly favourable water—these organisms multiply to a surprising extent and form a heavy growth on the larvae. No apparent harm is done, but it has been noticed that in such cases the larva very frequently fails to pupate successfully or dies early in the pupal stage.

Several larvae of *A. simpsoni* have been found heavily infected with the fungus *Coelomyces*, the body being a bright golden colour and completely packed with spores. This parasite, which attacks *Anopheles gambiae* and *A. funestus* in all stages, has not yet been found in the adult *A. simpsoni*.

Having regard to these and other factors which may influence the output of adults, it was decided that some attempt should be made to gauge this output in the case of one of the favourable plants—*X. sagittifolium*. The direct method used by Teesdale (1941)—*i.e.* caging the plant—was thought to have certain disadvantages. The first of these was that in spite of considerable care Teesdale had much trouble with predators (mantids and spiders) which hatched in his cages or found their way in from the outside. Further, the net method allows the study only of these larvae and pupae which were present in the axils at the time of enclosure.

Mr. C. B. Symes, then Senior Entomologist, Kenya Colony, suggested that a very fair estimate of the output of a plant could be made by collecting the pupae, leaving the larvae untouched. As this method seemed suitable, the following experiment was made :

Ten large plants in a plot at Bundibugyo were chosen, and all their axils were examined daily for 30 days. Records were kept of the numbers of axils and of the numbers with water, with larvae and with pupae. Larvae were not counted or handled in any way. Pupae were picked out in a pipette and taken to the laboratory for rearing ; an equivalent amount of water was returned to the axil in each case. Mosquito pupae are delicate and intolerant of handling, and a small number died during transit to the laboratory. These were identified as to species and sex (except in the case of 6 badly-damaged specimens of which the sex could not be determined). The remainder were reared out. The results are shown in Table VIII.

TABLE VIII.

Results of a daily collection of pupae from 10 *X. sagittifolium* plants at Bundibugyo.

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Totals	Means
Axils	69	69	69	69	69	70	71	73	72	72	72	73	73	73	73	73	74	73	73	73	74	74	73	73	73	72	72	72	73	73	—	72
Axils with water	46	47	48	45	47	40	44	46	46	46	57	57	58	58	58	58	58	59	59	58	57	57	57	55	55	56	57	57	57	60	—	53
Axils with larvae	30	29	31	31	31	35	36	36	37	37	40	41	44	48	49	49	49	50	50	50	49	49	49	49	49	49	51	51	51	53	—	43
Axils with pupae	4	3	0	4	1	3	4	1	3	5	3	3	3	5	2	4	2	2	7	3	2	4	2	2	2	3	2	3	4	6	—	3
Number of pupae	5	3	0	8	1	3	4	1	3	5	3	3	3	8	2	5	2	2	7	3	3	4	2	2	2	4	4	5	5	6	108	4
Species and sex of pupae																																
U. ornata v. *musarum*—																																
Males	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	2 } 3	—
Females	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1	
A. (S.) simpsoni—																																
Males	3	2	—	4	1	1	1	—	2	2	1	2	1	5	2	3	1	2	4	1	1	—	1	—	1	2	1	1	1	6	52 } 95	—
Females	2	1	—	3	—	2	3	1	—	2	2	1	1	3	—	2	1	—	2	2	—	2	1	—	1	1	1	3	2	—	39	
Sex not determined ...	—	—	—	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1	—	—	—	—	4	
E. chrysogaster group—																																
Males	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	2 } 8	—
Females	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1	—	—	2	—	—	—	4	
Sex not determined ...	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	2	
E. leucopus subsp. *productus*																																
Males	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1 } 2	—
Females	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1	

It will be noted that during the month the number of axils fluctuated between 69 and 74, as many new leaves opened while old ones died off. It was in order that this factor might be allowed for, that whole plants rather than a fixed number of axils were chosen as a unit. The rather larger fluctuations in the numbers of axils with water were due to the fact that all axils were counted, even those enclosed by old withered petioles—capable of retaining only a few drops of water for a short period after a shower. It will be noted that on the whole the numbers of axils with water and of axils with larvae increased during the month with the growth of the plants.

The results show a high and constant output of pupae. Every plant contained water and larvae on every day of the survey, and pupae were taken on 29 of the 30 days. In only about a dozen cases were 2 pupae found in a single axil, and in no case were more than 2 found together. The total of 108 pupae is high indeed when the very small size of the plant sample is taken into account. An average plot covering an acre might contain about 500 plants and, applying the foregoing figures, it is concluded that such a plot might produce about 5,000 *A. simpsoni* per month under favourable conditions; the number of females would be about 2,000. The output of a mixed plantation must be huge, when it is remembered that, apart from *Xanthosoma*, various other plants (gonja, pineapple, etc.) all add their quota. This, indeed, is shown by the fact that captures of up to 200 female *A. simpsoni* per day have been made on 5 successive days in a single plantation (ground covered by the catchers about 5 acres) without apparent diminution in the population of these mosquitoes.

Finally, to give some idea of the relative prevalence of the various axil-breeders in Bwamba as a whole, the total figures for all plants may be combined as follows:

Total axils examined	34,752
Axils with water	14,047
Axils with larvae	6,373
Percentage of potential foci inhabited	45
Aëdes (*Stegomyia*) *simpsoni*	10,679
Culex (*Culex*) *musarum*	10,040
Uranotaenia ornata var. *musarum*	3,171
Culex (*Culiciomyia*) *nebulosus*	648
Harpagomyia taeniarostris	584
Eretmapodites chrysogaster group	393
Eretmapodites ferox	227
Uranotaenia sp. incog.	128
Eretmapodites leucopus subsp. *productus*	85
Aëdes (*Stegomyia*) *aegypti*	29
Culex sp. near *toroensis*	11
Culex sp. near *trifilatus*	7
Total Larvae	26,002

The Fauna of Plant Axils in Bwamba.

In conclusion, a note may be given of some of the commoner inhabitants of plant axils, apart from mosquito larvae.

Holotrichous and peritrichous ciliates, diatoms, mobile unicellular algae and yeasts are all abundant and probably form a large proportion of the diet of mosquito larvae. Small free nematodes are quite common and planarians also occur. In large axils small aquatic oligochaetes live in the débris at the bottom.

Among insects, the larvae of Psychodids (subfamily PSYCHODINAE) are particularly abundant. Very occasionally small beetle larvae have been taken.

Small frogs ("tree-frogs") are quite common in axils. Experiments have shown that *A. simpsoni* feeds on these freely, and *vice versa*. In *Xanthosoma* axils various species of *Hyperolius* may be found, the commonest species in Bwamba closely resembling *H. picturatus* and *H. flavoviridis* (final determination is very difficult). An unidentified *Arthroleptis* has also been taken, and occasionally the peculiar terrestrial species, *Hemisus marmoratus*, may be found in axils at ground level. Pineapple axils are the haunt of a very beautiful little frog, *Megalixalus fulvovittatus*. This cream-coloured species has a neat pattern of longitudinal brown stripes, which form a highly effective disruptive pattern. When at rest in an axil this frog is very perfectly camouflaged. Banana axils have much the same fauna, including most of the species, and in addition *Kassina senegalensis* and *Leptopelis johnstoni*.

Summary.

1. A study of mosquito breeding in plant axils was carried out in Bwamba County, an area with much heavy forest in the extreme west of Uganda. Attention was directed mainly to *Aëdes* (*Stegomyia*) *simpsoni*, a known vector of human yellow fever in Bwamba.

2. The various plants studied are described.

3. The results of preliminary surveys are discussed. These confirmed that, as far as mosquito breeding is concerned, the most important plants in Bwamba are the lowland colocasia (*Xanthosoma sagittifolium*), the "gonja" variety of cultivated banana and the pineapple (*Ananas comosus*). It was shown that *A. simpsoni* is the commonest axil-dwelling larva in the inhabited lowland areas, and that it occurs in widely different types of lowland country. Records are given of the occurrence of larvae of *Aëdes* (*Stegomyia*) *aegypti* in plant axils.

4. A survey of 12 different kinds of plants is described, and it is shown that the larval populations depend to a considerable extent on the amounts of water retained by the axils of the various plants concerned. In Bwamba, medium-sized axils with a capacity of 4 to 8 cc. appear to be the most favourable. Certain plants which yielded large numbers of larvae—such as the wild banana (*Musa* sp.)—were not found to harbour *A. simpsoni*. Apparently this is due to the fact that they are confined to forest and mountain areas, where *A. simpsoni* is scarce or absent.

5. A monthly survey of *Xanthosoma* axils, carried out over a period of 12 months, showed that *A. simpsoni* larvae were continually present and that the fluctuations in larval population were dependent on growth and harvesting of the plants rather than on rainfall.

6. Scrapings from dry *Xanthosoma* axils, taken during a short period of drought, were placed in bowls of water in mosquito-proof cages. Larvae of *A. simpsoni* and *Harpagomyia taeniarostris* hatched from this material in 2 to 3 days.

7. Temperature measurements in a *Xanthosoma* axil showed that the water therein was cooler than the surrounding air and also that the axil temperature was remarkably equable.

8. Systematic collections of pupae from *Xanthosoma* axils showed that the mosquito output (which cannot be gauged directly from the population of larvae) is high and constant. It is estimated that a plot of 500 plants of this species may, under favourable conditions, produce something like 5,000 *A. simpsoni* (2,000 females) per month.

The influence of predatory larvae (*Eretmapodites* spp.) on the populations of other species is thought to be considerable.

9. The fauna of plant axils, apart from mosquito larvae, is described briefly.

Acknowledgements.

I am indebted to Mr. G. H. E. Hopkins, O.B.E., Senior Entomologist (Medical), Uganda Protectorate, Mr. J. O. Harper, Kenya Medical Dept., Mr. J. D. Gillett, Uganda Medical Dept., and the late Mr. E. G. Gibbins, also of the Uganda Medical Dept., for access to unpublished records of plant-axil and small-container larvae. Mr. A. S. Thomas, Dept. of Agriculture, Uganda, has given much useful information about the plants concerned. The careful work of my African Assistant, Om. Y. Senkubuge, deserves special mention.

References.

BUXTON, P. A. & HOPKINS, G. H. E. (1925). Race suicide in *Stegomyia*.—Bull. ent. Res., **16**, pp. 151–153.

—— & ——. (1927). Researches in Polynesia and Melanesia. Parts I–IV.—Mem. Lond. Sch. Hyg. trop. Med., no. 1. London.

DUNN, L. H. (1926). Mosquitos bred from dry material taken from holes in trees.—Bull. ent. Res., **17**, pp. 183–187.

——. (1927). Tree-holes and mosquito breeding in West Africa.—Bull. ent. Res., **18**, pp. 139–144.

GIBBINS, E. G. (1942). On the habits and breeding-places of *Aëdes* (*Stegomyia*) *simpsoni* Theobald in Uganda.—Ann. trop. Med. Parasit., **36**, pp. 151–160.

HADDOW, A. J. (1945a). On the mosquitoes of Bwamba County, Uganda. I. Description of Bwamba with special reference to mosquito ecology.—Proc. zool. Soc. Lond., **115**, pp. 1–13.

——. (1945b). The mosquitoes of Bwamba County, Uganda. II. Biting activity with special reference to the influence of microclimate.—Bull. ent. Res., **36**, pp. 33–73.

——. (1945c). The mosquitoes of Bwamba County, Uganda. III. The vertical distribution of mosquitoes in a banana plantation and the biting cycle of *Aëdes* (*Stegomyia*) *simpsoni* Theo.—Bull. ent. Res., **36**, pp. 297–304.

——. (1946). The mosquitoes of Bwamba County, Uganda. IV. Studies on the genus *Eretmapodites* Theobald.—Bull. ent. Res., **37**, pp. 57–82.

——, SMITHBURN, K. C., MAHAFFY, A. F. & BUGHER, J. C. (1947). Monkeys in relation to yellow fever in Bwamba County, Uganda.—Trans. R. Soc. trop. Med. Hyg., **40**, pp. 677–700.

HOPKINS, G. H. E. (1936). Mosquitoes of the Ethiopian Region. I. Larval bionomics of mosquitoes and taxonomy of Culicine larvae.—London, Brit. Mus. (Nat. Hist.).

MAHAFFY, A. F., SMITHBURN, K. C., JACOBS, H. R., & GILLETT, J. D. (1942). Yellow fever in western Uganda.—Trans. R. Soc. trop. Med. Hyg., **36**, pp. 9–20.

SHANNON, R. C. (1931). The environment and behaviour of some Brazilian mosquitoes.—Proc. ent. Soc. Wash., **33**, pp. 1–27.

SMITHBURN, K. C. & HADDOW, A. J. (1946). Isolation of yellow fever virus from African mosquitoes.—Amer. J. trop. Med., **26**, pp. 261–271.

TEESDALE, C. (1941). Pineapple and banana plants as sources of *Aëdes* mosquitoes.—E. Afr. med. J., **18**, pp. 260–267.

TOTHILL, J. D. Ed. (1940). Agriculture in Uganda.—London, Oxford Univ. Press.

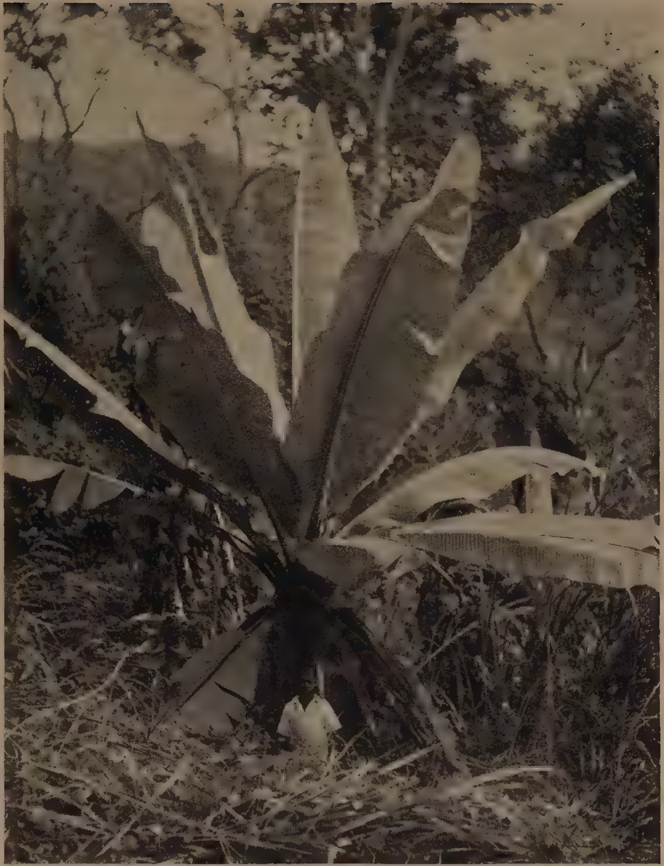

Fig. 1. Wild banana or " kitembe " growing in the Ruwenzori foothills at 6,000 to 7,000 feet. Note the enormous leaves and short " stem." The boy—Babwizi tribe—is about 4 feet 8 inches high.

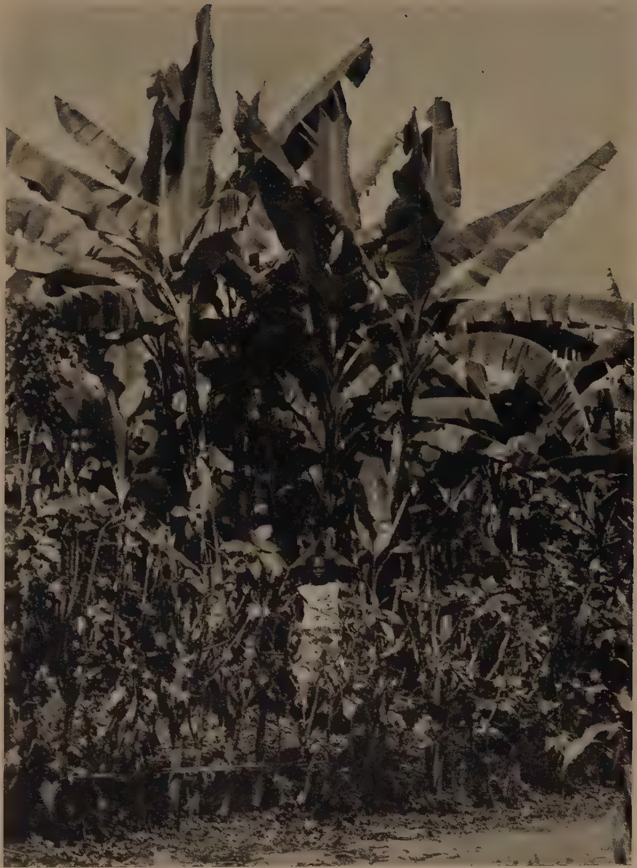

Fig. 2. A group of bitoke bananas (" namunu " in Bwamba) of the variety locally known as " njabalima." Note the blackish blotches on the upper part of the stems and the small number of leaves per plant. The boy—Bambuba tribe—is about 5 feet 3 inches high.

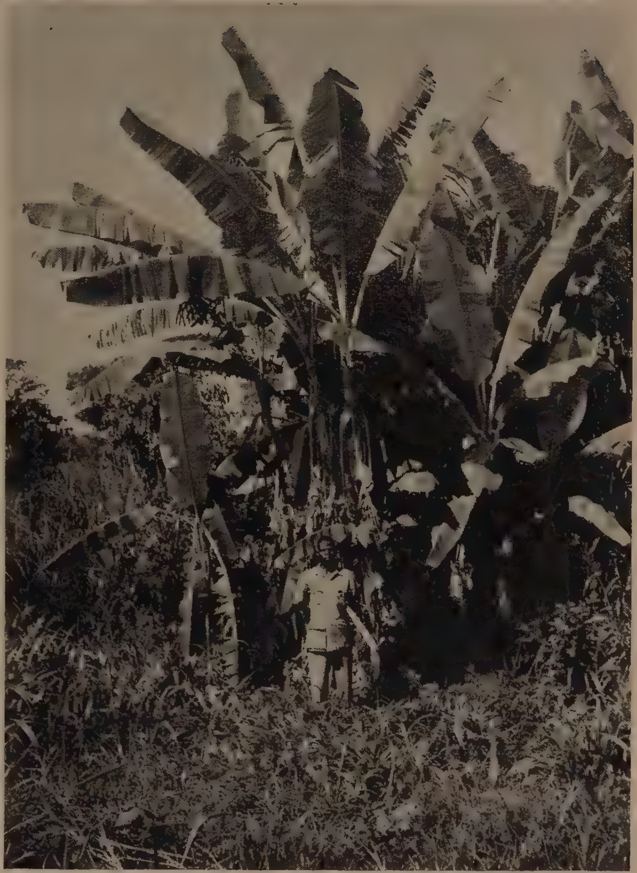

Fig. 1. A group of menvu bananas of the variety known locally as "kasukali." The fruits of this type are yellow-skinned. Note the blotched stems, the small number of leaves per plant and the persistent withered leaves hanging downwards. The boy—a Muganda—is about 5 feet 8 inches in height.

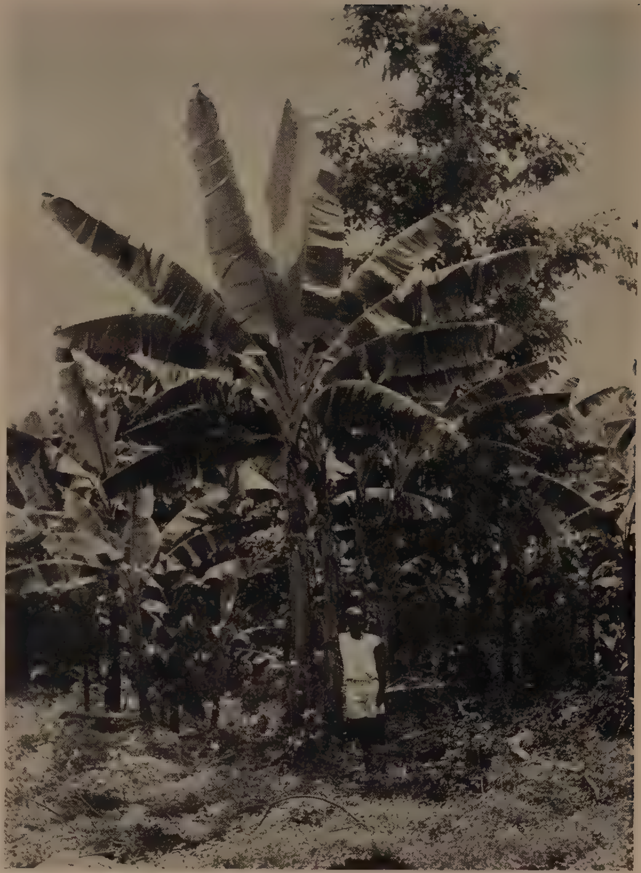

Fig. 2. A small group of gonja bananas ("bikonje" in Bwamba) of the variety locally known as "kikonjekonje." Note the well-formed axils sheathing the stem, the light colour of the stem and the large number of leaves in the crown. The smaller plants in the background are recently planted bitoke bananas. The boy—Baburaburi tribe—is about 4 feet 8 inches high.

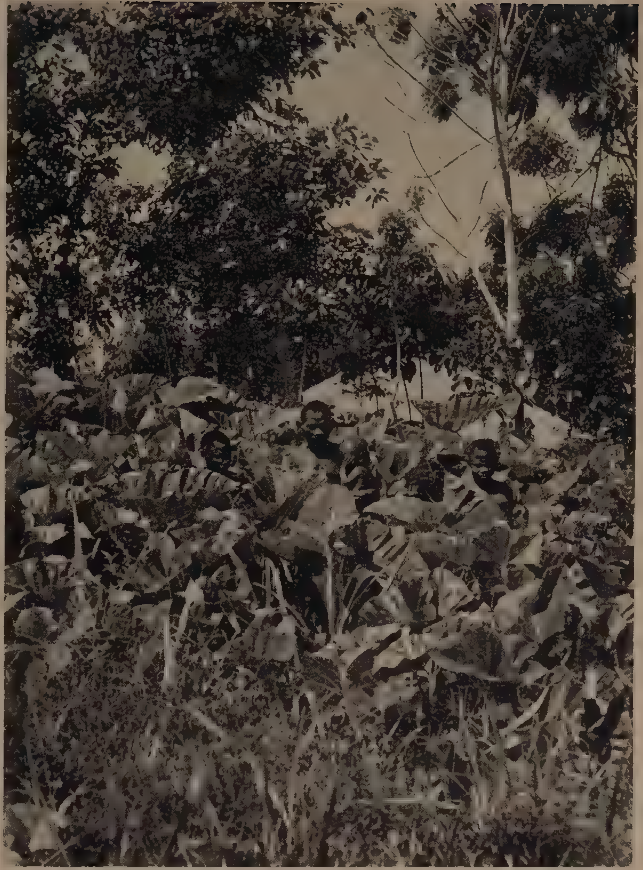

Fig. 1. A typical plot of the green-stemmed lowland colocasia (*Xanthosoma sagittifolium*, Schott.), known locally as " binongwe byekijungu." The people are Bambute pygmies, the man being about 5 feet in height.

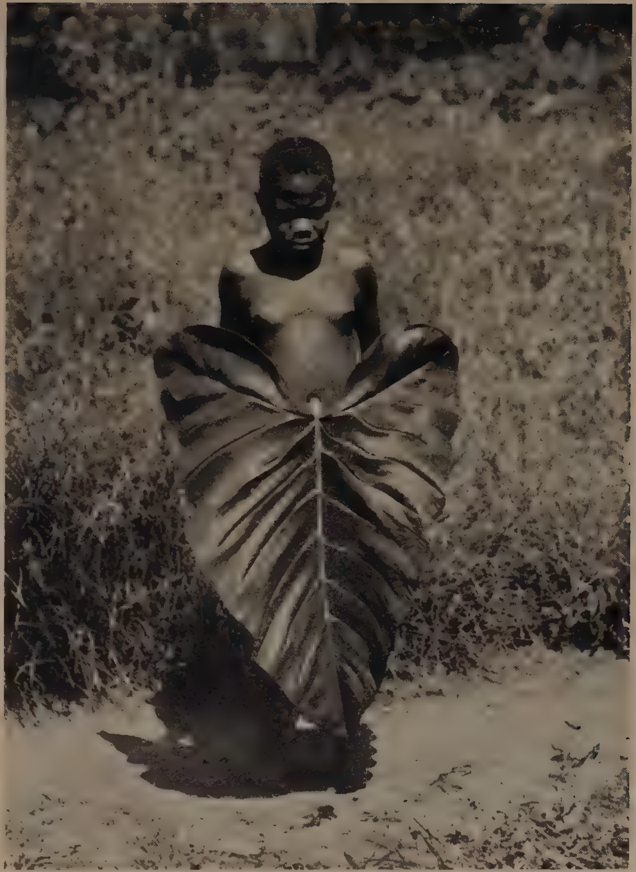

Fig. 2. A single leaf of *X. sagittifolium*, showing the large size and hastate outline. The woman is a Mumbute pygmy, just over 4 feet in height.

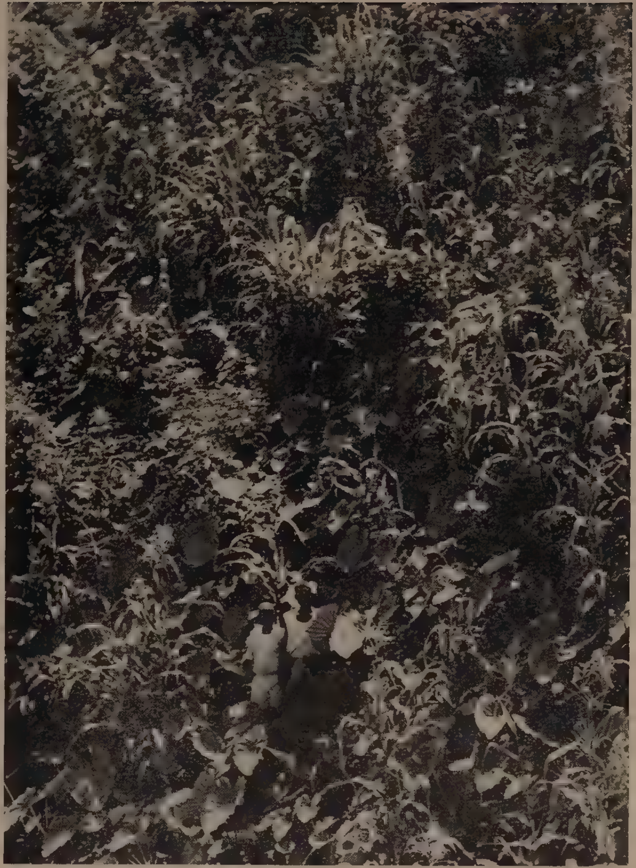

Fig. 1. A plot of the brown-stemmed highland colocasia (*Colocasia esculentum*, Schott.), locally known as "binongwe byekikonjo," growing at about 5,500 feet in the Ruwenzori foothills. Note the admixture of maize plants. The taller boy—Babwizi tribe—is about 5 feet 3 inches in height.

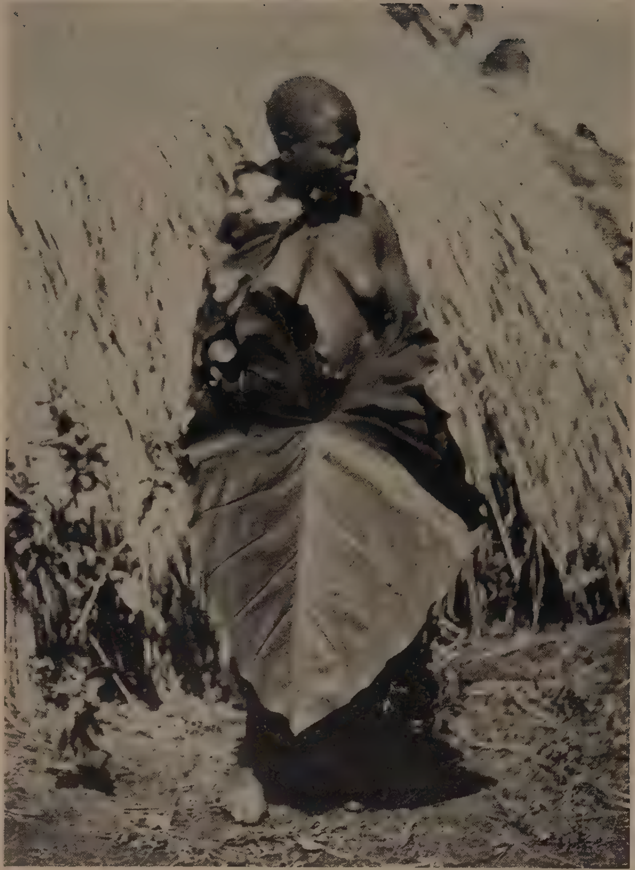

Fig. 2. A single leaf of *C. esculentum*, showing the large size and peltate outline. The woman, a Mukonjo of the mountain tribe, is about 5 feet in height.

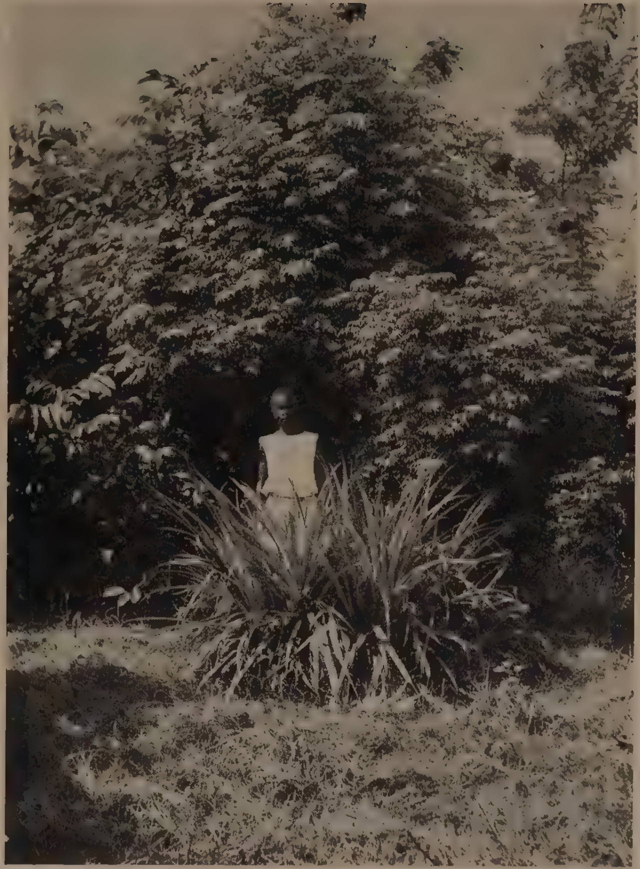

Fig. 1. A cluster of pineapples (locally known as " kinanansi ") growing at the edge of a mixed *Melea—Markhamia* plantation. The boy—Babwizi tribe—is about 5 feet in height.

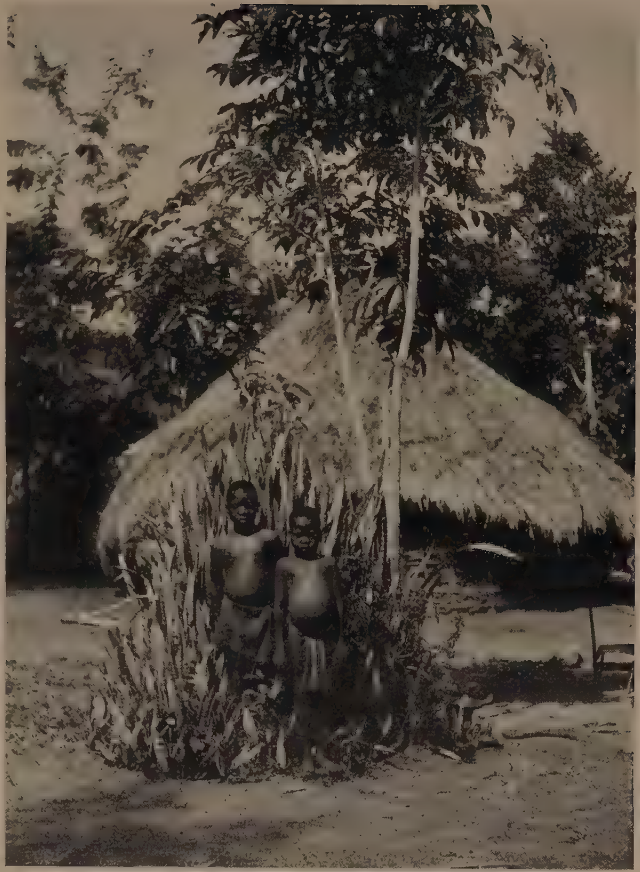

Fig. 2. A clump of " manusi " (*Sansevieria* sp.) in a hut clearing. The taller boy—Babwizi tribe—is about 4 feet in height.

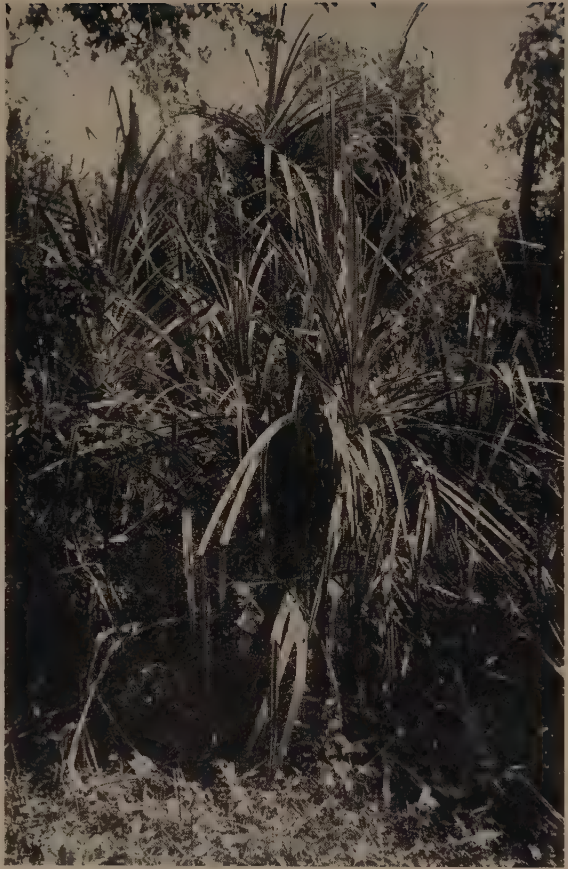

Fig. 1. Small *Pandanus* plants (" kigagara ") growing at the edge of a thicket in the forest. The boy—Babwizi tribe—is about 5 feet 8 inches in height.

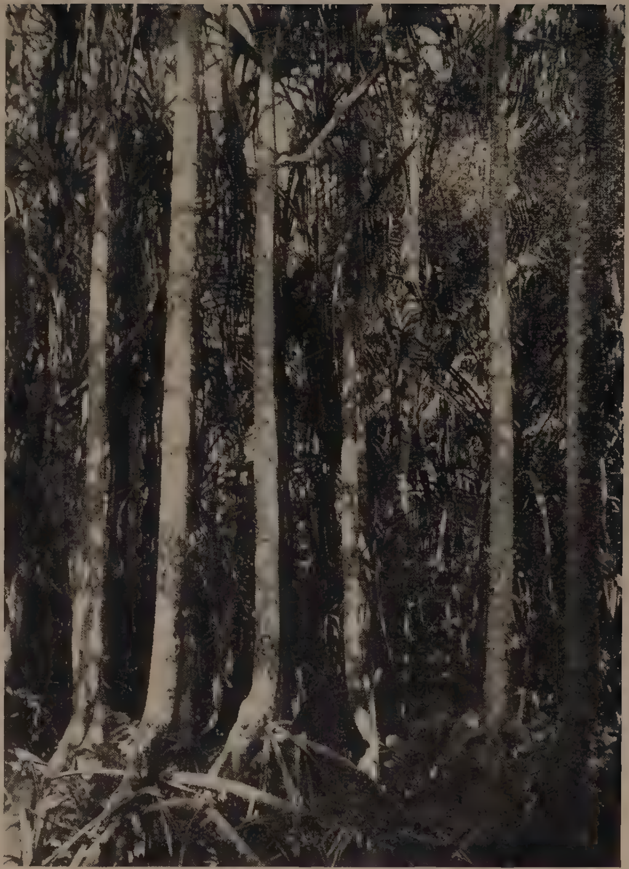

Fig. 2. Large *Pandanus* plants in the middle of the same thicket.

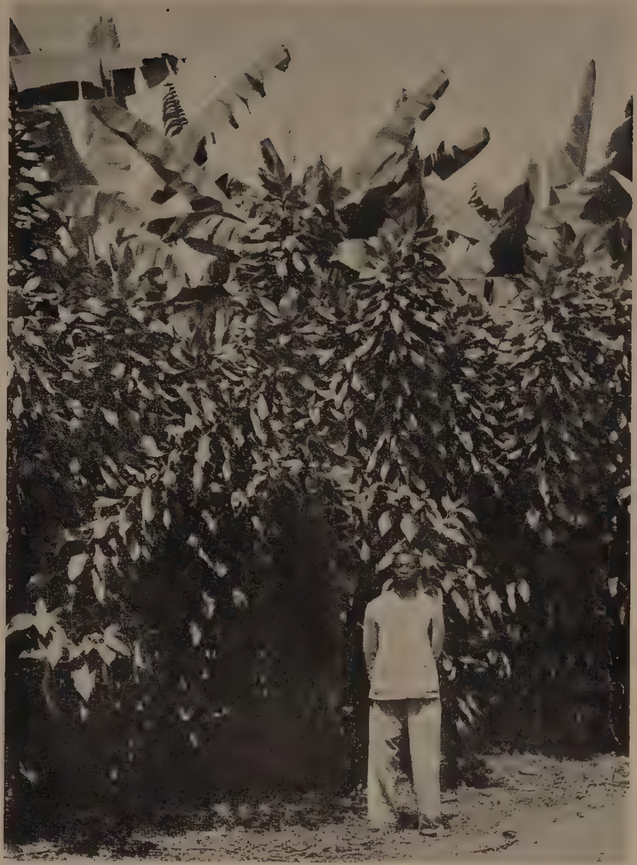

Fig. 1. A hedge of *Dracaena ugandensis* ("karamura") surrounding a bitoke plantation. The man—a Muganda—is about 5 feet 8 inches in height.

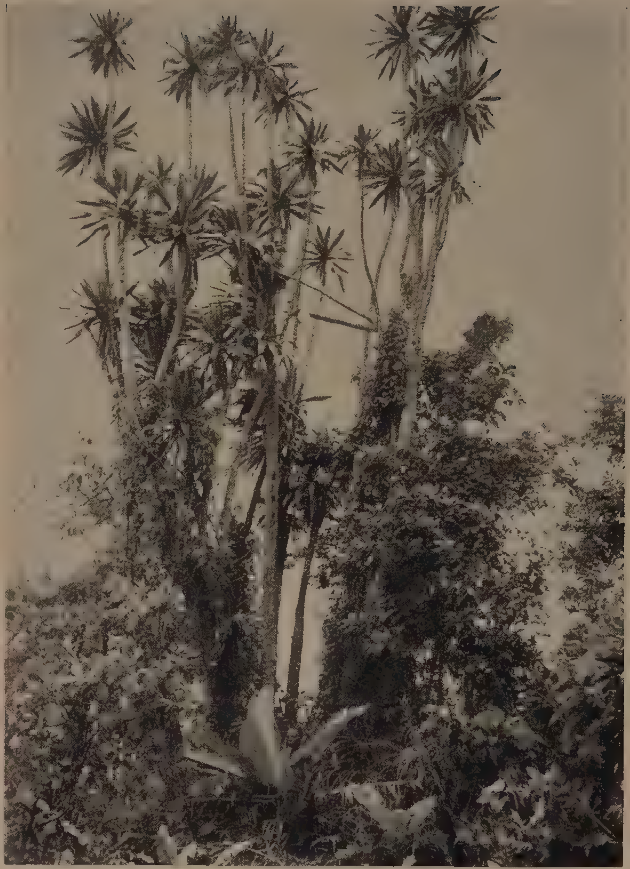

Fig. 2. A large *Dracaena steudneri* ("kagorogoro") in the Ruwenzori foothills. The boy—Bakonjo tribe—is about 5 feet 6 inches in height.

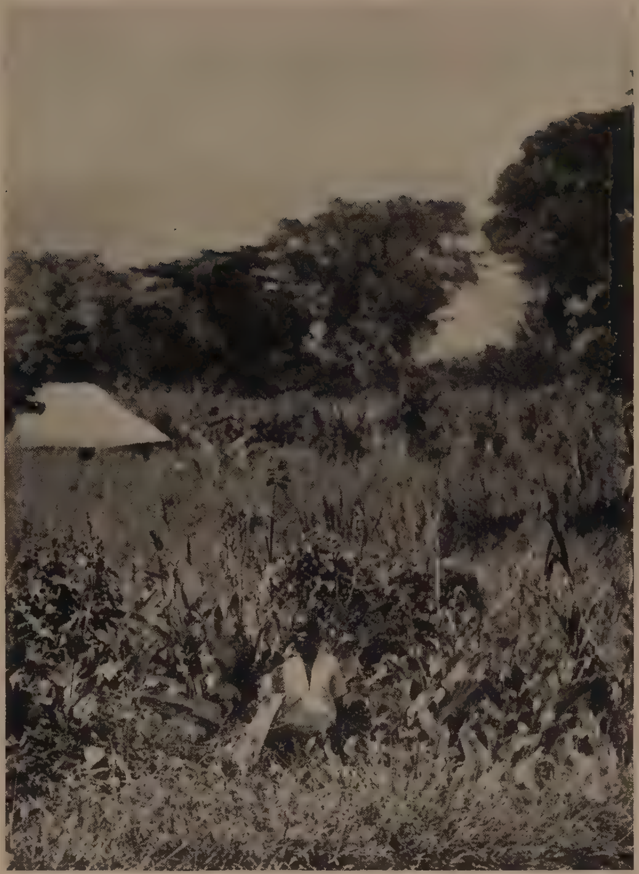

Fig. 1. A patch of *Canna orientalis* (" maranga ") growing wild by the roadside. The boy—Babwizi tribe—is about 5 feet 8 inches in height. He is standing in the ditch at the point where the cannas spring from the ground.

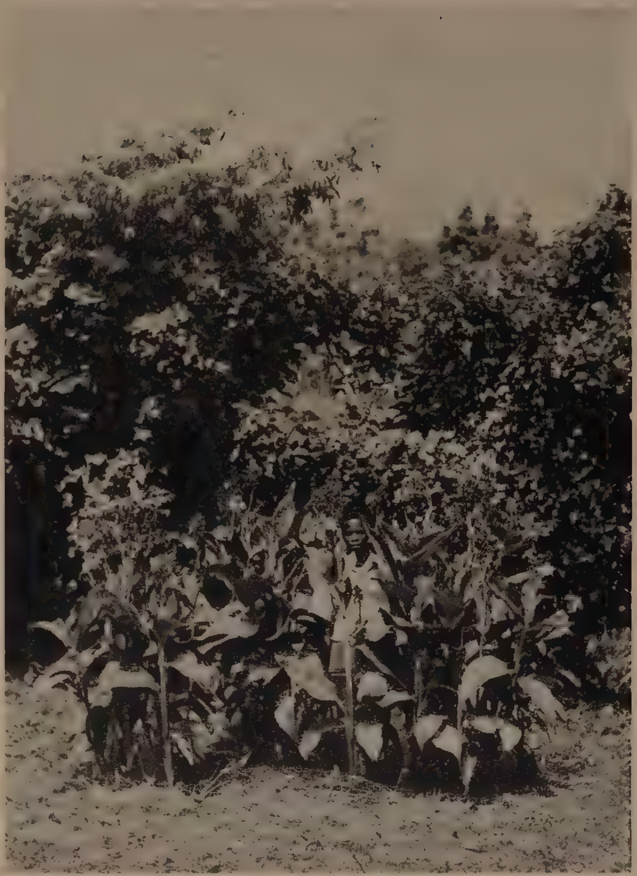

Fig. 2. A clump of cannas (large garden hybrid) growing in a native garden. The boy—Babwizi tribe)—is about 5 feet 6 inches in height.

EFFECT OF DEFOLIATION ON REPRODUCTION OF *CORDIA MACROSTACHYA*.

By E. McC. Callan.

Entomology Department, Imperial College of Tropical Agriculture, Trinidad, B.W.I.

Cordia macrostachya (Jacq.) Roem. & Schult. is a native of tropical America and was probably introduced into the island of Mauritius about 1890 from British Guiana. Since then the plant has spread rapidly over the island and become a weed; it is now so serious that an investigation of its insect fauna has been made in its native environment with a view to controlling it by introducing its insect enemies into Mauritius. Donald (1945 unpublished) and Wiehe (1946) have reported on this work, which has been carried out at the Imperial College of Tropical Agriculture in Trinidad, B.W.I.

The most promising insects for introduction into Mauritius are two species of phytophagous beetles, *Physonota alutacea*, Boheman, and *Schematiza cordiae*, Barber, which are very destructive to the foliage and appear to be specific to the plant. Wiehe (*loc. cit.*) has pointed out that in Mauritius the weed can withstand a considerable amount of defoliation and has suggested that these leaf-eating beetles might not inflict sufficient damage to reduce its aggressive power. On the other hand, it is not unlikely that they may reduce the reproductive capacity of the plant sufficiently for it to become a subordinate instead of a dominant species. It is therefore desirable to obtain evidence on this point before their introduction into Mauritius is attempted.

In the experiments described below it was unfortunately not possible to infest the plants with enough beetles to produce defoliation. The leaves were therefore removed by hand. While admitting that this is an obvious defect in the experiments, the results are sufficiently striking to show that defoliation has a significant effect in reducing the reproduction of the plant. In Trinidad these beetles are heavily parasitised and it remains to be seen whether in a new environment and freed from their parasites they can bring about sufficient defoliation to affect reproduction.*

Experimental Technique.

The experiments were carried out from October to December 1945 in an abandoned field at St. Augustine. Nine plots were chosen at random in each of which four plants as nearly as possible comparable in size were selected. The plants were probably about two years old and were on an average five feet in height.

At weekly intervals three plots (Nos. 1–3) were defoliated by hand. The plants were not completely stripped of leaves, only the larger ones about 10 to 15 cm. in length being removed. During eight weeks 10,600 leaves were removed from these plots or an average of 110 leaves per plant per week. The six remaining plots (Nos. 4–9) were left as controls.

An attempt was made to determine (1) the rate at which new inflorescences were produced by defoliated and control plants, and (2) the number of fruits formed on these inflorescences. To find the rate at which new inflorescences were produced, all young ones about 2 cm. in length were marked with differently coloured wool at weekly intervals. The age of the inflorescences on a plant could be seen at a glance and the number produced in a given time readily determined.

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* Dr. F. J. Simmonds of the Commonwealth Bureau of Biological Control, who has now taken over this investigation, has recently obtained evidence (Simmonds, 1948) that *Schematiza cordiae*, Barber, in the absence of its parasites, is capable of causing marked defoliation in Trinidad.

A random sample of fully formed inflorescences was also selected each week. Measurements were made of their length (excluding the peduncle) and the numbers of flower-buds and fruits were counted. There were usually relatively few open flowers at any one time on an inflorescence, but these were recorded with the unopened flower-buds. All set fruits were counted. The vast majority of these were green unripe fruits and only an occasional fruit ripened during the experiments. Such ripe fruits were counted with the unripe fruits.

Results.

Tables I and II show the results obtained from the defoliated and control plots.

TABLE I.

Production of inflorescences by 12 defoliated trees (Plots Nos. 1–3) in 8 weeks.

Plot No.	No. of leaves removed	No. of new inflorescences	Length of inflorescences in cm.	No. of flower-buds	No. of fruits
1	4,425	79	5·1	1,695	103
2	3,050	39	4·7	538	59
3	3,125	73	5·3	393	32
Total ...	10,600	191	—	2,626	194

TABLE II.

Production of inflorescences by 12 control trees (Plots Nos. 4–9) in 8 weeks.

Plot No.	No. of new inflorescences	Length of inflorescences in cm.	No. of flower-buds	No. of fruits
4	129	8·35	2,792	504
5	115	10·1	3 095	1,005
6	170	9·55	2,676	960
7	158	8·3	1,619	245
8	124	8·4	1,566	678
9	163	8·9	1,780	721
Total	859	—	13,528	4,113

The average production of inflorescences per defoliated plant was 15·9 and per control plant 35·8 in eight weeks. This shows clearly the effect of defoliation on the production of inflorescences.

The average length of the inflorescences on the defoliated plots was 5·0 cm. and on the control plots 8·9 cm. The average production of flower-buds and fruits per defoliated plant was 219 and 16·2 respectively while the equivalent production per control plant was 564 and 171 in eight weeks. The effect of defoliation on the length of the inflorescences and on the production of flower-buds and fruits is clearly indicated. The results are especially striking as regards fruit production, the control plants producing more than ten times as many fruits per plant as the defoliated plants.

Conclusions.

The results of the above experiment show that defoliation by hand has a marked effect on the plant. The rate of production of new inflorescences is reduced and those that are formed are shorter and bear fewer flower-buds and fruits. The production of fruits is especially reduced, probably as a result of depletion of food reserves. As the plant appears to reproduce entirely by seed, it can be concluded that defoliation has a significant effect in reducing its reproductive capacity.

It is interesting to note, incidentally, that these results support the generalisation that the defoliation of crop plants by leaf-eating insect pests has an adverse effect on the plants which may be especially marked as regards fruit production.

Summary.

The importance of finding the effect of defoliation on the reproductive capacity of *Cordia macrostachya* is explained in view of the proposed introduction of two species of leaf-eating beetles from Trinidad into Mauritius for the control of this weed.

An account is given of experiments in which the production of inflorescences and fruits was compared in defoliated and control plants.

The results show that defoliation has a significant effect in reducing the reproductive capacity of the plant.

References.

Simmonds, F. J. (1948). An illustration of effective control exerted by parasites on their host.—Bull. ent. Res., **39,** pp. 217–220.

Wiehe, P. O. (1946). Report on a visit to Trinidad, Louisiana and other countries.—Publ. Mauritius Government, no. 28, 119 pp.

THE EFFECTIVE CONTROL BY PARASITES OF *SCHEMATIZA CORDIAE*, BARBER, IN TRINIDAD.

By F. J. Simmonds, M.A., B.Sc., Ph.D.
Commonwealth Bureau of Biological Control.

Cordia macrostachya (Jacq.) R. & S. (Boraginaceae) is a serious pest in Mauritius, to which island it was accidentally introduced from the West Indies. During investigations of the possibility of the biological control of this weed, Kirby (1944 unpublished report, "Insects of *Cordia* in Trinidad ") and Donald (1945 unpublished dissertation) worked out the biology of the Galerucid, *Schematiza cordiae*, Barber (J. Wash. Acad. Sci. **37**, p. 243, 1947), a defoliating species that gave promise of meriting consideration for introduction into Mauritius.

In 1946, further investigations in Trinidad on this species were started. It is widespread over the island and occasionally damages much of the leaf area of a *Cordia* plant, both adults and larvae feeding on the leaves. Individual leaves are not eaten entirely, but different amounts of skeletonising occur, and this is particularly severe on the small tender shoots of small *Cordia* bushes. Although, however, the beetle is widespread and common, it is seldom abundant enough to produce really serious damage to the host plants.

TABLE I.

Parasitism of the immature stages of *Schematiza* sp. in Trinidad.

A. Egg stage	No. of eggs in collections	No. of *Schematiza* larvae emerging	No. of *Tetrastichus* emerging	Apparent percentage parasitism
	1,337	215	1,122	83·9

Larval stages	No. of larvae in collections	No. un-parasitised	No. parasitised by *Chaetonodexodes*	No. parasitised by *Chaetonodexodes* and *Spilochalcis*	Percentage parasitised	No. of those hyper-parasitised
1st stage	158	48	110	—	69·6	0·0
2nd stage	341	47	294	4	86·2	1·4
3rd and 4th stages	168	28	140	11	83·3	7·9

In the laboratory the adults are long-lived (up to ten weeks) and may produce some 1,000 eggs per pair. The reproductive potential of the species is enormous, and, moreover, it is tolerant of the fairly wide range of environments found in Trinidad. There must, therefore, be some factor or factors checking rapid increase in the population of *Schematiza*. Observations in the field indicate that predacious wasps (principally *Polistes canadensis*, L.) may take some of the larvae ; ants and insectivorous birds may also be responsible for some mortality. Field collections of the immature stages show that parasites certainly account for most of the destruction of the eggs and larvae. The figures given in Table I, the totals of a number of collections, show the percentages of eggs and larvae destroyed by parasites. Samples taken at intervals over a wide area indicate that the figures given here are representative of the degree of parasitism occurring generally in the island. The eggs are parasitised by a new species of *Tetrastichus* (Eulophidae, Hymenoptera) and the larvae may be attacked from the time of emergence until possibly the end of the second stage by a larvipositing Tachinid, *Chaetonodexodes marshalli*, Aldr., superparasitism being very common. Within the *Schematiza* larva a single Tachinid larva feeds and grows, but it does not reach the second stage until the host has ceased feeding. Before this occurs the Tachinid larva may itself be parasitised by *Spilochalcis* sp. (Chalcididae, Hymenoptera). This hyperparasite of *Schematiza* attacks the parasitised larvae of the latter and lays an egg within the body of the Tachinid larva. The egg does not hatch until after the primary host has become full-grown. There are other parasite species attacking *Schematiza*, but those described above are the commonest and are the species mainly concerned in the reduction of its numbers.

In conjunction with some other experiments an illustration was obtained of the destruction of *Cordia* leaves which might be accomplished by *Schematiza* in the absence of parasites. Six large cages were made, suitable for placing over individual bushes of *Cordia* (see fig. 1). These cages consisted of a strong wooden frame 4½ feet high and 3 feet in length and width. On one side was a hinged door, and at the bottom of the four corners were wooden spikes that could be driven into the ground. Three of these cages were covered, sides, door and top, with half-inch mesh chicken-wire.

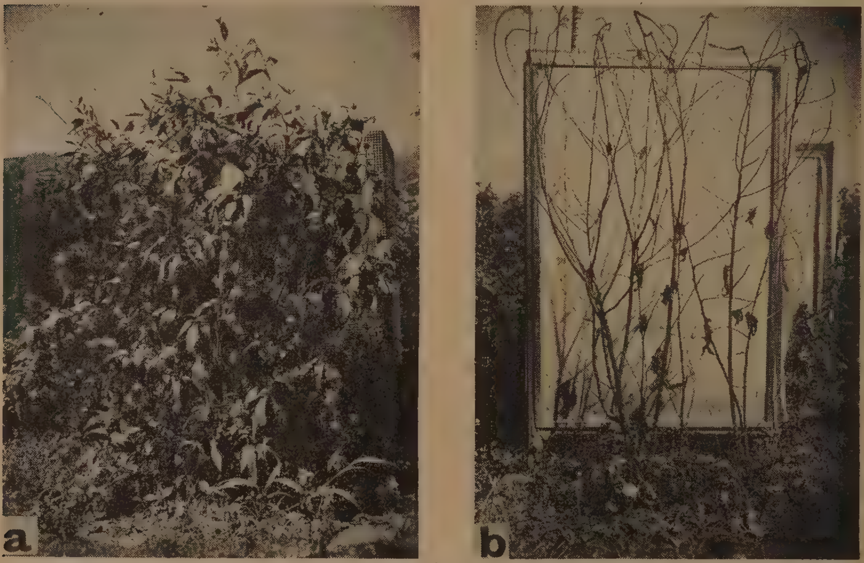

Fig. 1.—Effect on the host plant, *Cordia macrostachya*, of the elimination of natural enemies of a defoliating beetle, *Schematiza cordiae*, Barber.

a. *Cordia* bush after 8 weeks in chicken-wire covered cage.

b. *Cordia* bush after 8 weeks in a cage covered with cheese-cloth. For the last 3 weeks exposed to *Schematiza* freed from the control of parasites.

The remaining three were covered similarly with fine cheese-cloth. In an area covered with *Cordia* bushes about 4 feet high a clearing was cut, leaving 6 bushes of similar size and growth fairly equidistantly spaced over it. The earth around these was dug to destroy other vegetation and a cage was placed over each bush, the spikes driven into the ground, and the sides earthed up at the bottom to eliminate gaps. After a month all six bushes had grown somewhat and were quite healthy, any changes in temperature and humidity under the cheese-cloth having had no apparent adverse effect on the plants. On 9th December a few *Schematiza* adults and egg masses were observed in all the cages. The cages were then left undisturbed until 31st December, when they were examined. It was found that the *Cordia* bushes under the three chicken-wire covered cages were healthy, whereas those under the cheese-cloth were completely defoliated, the leaves skeletonised by *Schematiza* having dropped off. This striking difference is shown in the two illustrations taken ten days later. The number of *Schematiza* larvae, pupae and adults in each cage covered with cheese-cloth was conservatively estimated at approximately 2,000–3,000. The larvae were crawling over both the inside and outside of the cages in large numbers in search of food, and the decaying leaves on the floor of the cages sheltered very many pupae. It is interesting to note, in connection with the specificity of *Schematiza* in its feeding habits, that weeds other than *Cordia* that had grown on the floor of the cage were practically untouched even when so many starving beetle larvae were present.

There appear to be only two factors that could account for the difference between the populations of *Schematiza* in the two types of cage and the consequent differences between the *Cordia* plants. One is that of the actual physical effect on the larvae of rain beating down on the bush. This was absent in the cages covered with cheese-cloth, but it is extremely unlikely to be responsible for the observed differences. The other factor is the absence of natural enemies from these cages, excepting some ants which were present in all cages. The action, if any, of birds in reducing the numbers of *Schematiza* was eliminated from all the cages, but both insect predators and parasites could, and did, enter the cages covered with chicken-wire, but were excluded from those covered with cheese-cloth. Thus the conclusion to be drawn is that the absence of predators and parasites, particularly the latter, is responsible for the amazingly rapid increase of *Schematiza*. Further support is, of course, given to this by the figures for parasitism given above. Also, when the cages were removed from the plants a very high concentration of larvae and adults of *Schematiza* was found in the immediate vicinity of the cages. Considering the number of adults present, comparatively few young larvae were found subsequently, and the collections of egg masses showed that parasitism was nearly as high as in " control " collections made at some distance from the cages. Furthermore, collections of young larvae near the cages showed parasitism of :—1st stage 100·0 per cent., 2nd stage 94·2 per cent. (3·1 per cent. of these parasitised by *Spilochalcis*), 3rd and 4th stages 81·8 per cent. (11·1 per cent. by *Spilochalcis*). The decline in parasitism is due to the numbers of already half-grown larvae freed from the cages. A control collection made some hundred yards from the cages gave :—1st stage 80·7 per cent., 2nd stage 93·6 per cent., 3rd and 4th stages 100·0 per cent. (12·5 per cent. by *Spilochalcis*). Egg masses collected at the same time gave parasitism of :—near to cages 39·9 per cent., away from cages 77·4 per cent. These figures are not very satisfactory as indications of parasitic activity since it is not known how many more eggs would have been parasitised had they not been removed from the field, nor the time that elapses between the laying of the eggs and the stage when they become suitable for parasitism. The indication is, however, that *Tetrastichus* cannot deal quickly with a sudden local increase in the numbers of *Schematiza* eggs, possibly owing to poor mobility of the adults. *Chaetonodexodes*, on the other hand, appears to be able to control local increases in numbers of host larvae very quickly, the flies probably congregating quickly at such points. Large numbers were seen flying around the bushes.

A very short time after the population of *Schematiza* had been thus artificially increased near the cages, the appearance of the *Cordia* plants showed clearly that natural enemies again had *Schematiza* well under control and that no serious damage would be done to the host plants. This shows the controlling effect of parasites on *Schematiza*; when this control is removed the population increases rapidly, but when it is resumed the population decreases quickly to its former level.

R

MOSQUITOS OF SOCOTRA.

By H. S. Leeson.
London School of Hygiene and Tropical Medicine,
and
O. Theodor.
Department of Parasitology, Hebrew University, Jerusalem.

In December 1942 and January 1943, the late Captain T. P. Ward, R.A.M.C., carried out a malaria survey of the island of Socotra accompanied by Private G. F. W. Hart, R.A.M.C., who collected the mosquito material. The following notes have been abstracted from Captain Ward's official report.

> “ The island of Socotra is situated in the Indian Ocean some 150 miles east of Cape Guardafui and 560 miles east of Aden. The island is roughly 80 miles long and about 25 miles wide and the area is roughly 2,000 square miles. There are coastal plains and a central range of mountains and plateaux between 1,500 and 4,700 feet altitude. Numerous water courses (wadis) intersect the mountains and plateaux and run in all directions down to the coastal plains. In the larger wadis from the high mountains there is much water, but the plateaux and coastal plains are generally arid. An exception is the Hadibo plain which is well watered, having three long shallow rivers with large lagoons at their mouths.
>
> “ The climate is subtropical, the day temperature seldom exceeding 90°F. in the shade. The night temperature is usually about 70°F. in the plains but in the mountains and plateaux often drops to 50°F. The north-east monsoon starts towards the end of November and continues to the end of January with heavy rain in December and January, but at the time of [Capt. Ward's] visit little rain had fallen. The south-west monsoon begins at the end of May and lasts until September. It is stronger than the north-east monsoon and often reaches gale force. Information obtained from the inhabitants is that much rain falls during this time, but there are no official records.
>
> “ The native population totals 6,000, Arab, Negro and Bedouin. The Arabs and Negroid types live mainly in the coastal plains in mud houses. The Bedouins live in the mountains and some of them on the coastal plains ; their dwellings are caves or stone houses, of which a large number are deserted. The incidence of malaria is high in all parts of the island particularly among the Bedouins, though December to January appears to be the quiescent period. Local information gives the fever season as spring and summer.
>
> “ With two exceptions intense Anopheline breeding was discovered in all water in wadis, rivers, lagoons and wells ; the exceptions were the lagoon at Khor Garrich on the north coast 13 miles east of Hadibo and a lagoon 6 miles south-west of Hadibo. In all other lagoons larvae were found at the edges among algae or where there was no vegetation at all, both in sunny and shaded situations. Fast running streams, clean mountain pools and dirty, stagnant water and practically all wells, shallow or deep, contained Anopheline larvae.
>
> “ Two species of *Anopheles* were found, *A. culicifacies* var. *adenensis* and another related to *A. dthali* which may be new. Culicines were found in many localities.”

The collection of mosquito material consists of 22 adults caught in houses, 300 adults bred from larvae and 100 samples of larvae preserved in spirit. There are no bred series containing adults with their respective larval and pupal pelts ;

consequently identifications have had to be made separately for adults and for larvae. Accordingly, the following list of species is provisional.

Anopheles (*Myzomyia*) *dthali wardi*, var. nov.
,, ,, *culicifacies* var. *adenensis*, Christ.
Aëdes (*Stegomyia*) *granti*, Theo.
,, ,, *vittatus*, Big.
Culex fatigans, Wied.
,, *sitiens*, Wied.
,, *thalassius*, Theo.
,, *laticinctus*, Edw.
,, *sinaiticus*, Kirkp.

No Anopheline had previously been recorded from Socotra. The only mosquitos mentioned by Edwards (1941) are *Aëdes* (*Stegomyia*) *granti* (female only) and *A.* (*Stegomyia*) *aegypti*, L. (which was not taken by Capt. Ward). According to Edwards (1941), the only records are those by Grant and Theobald in Forbes (1903) and by Becker (1910). According to Forbes, Socotra is geologically quite distinct from the mainland of Africa and Arabia. Its fauna is also distinct and *A. granti* is an isolated species not known to occur elsewhere.

This paper includes a description of the new variety of *Anopheles dthali* and of the hitherto unknown male and larva of *Aëdes granti*, and some notes on the other mosquitos.

Some of the material is deposited in the British Museum; the remainder is in the London School of Hygiene and Tropical Medicine.

ANOPHELINES.

Anopheles (Myzomyia) dthali wardi var. nov. (fig. 1).

Under low magnification the adult appears small and delicate and has a pale thorax and distinctly banded abdomen. It resembles *A. dthali* but differs in that the three

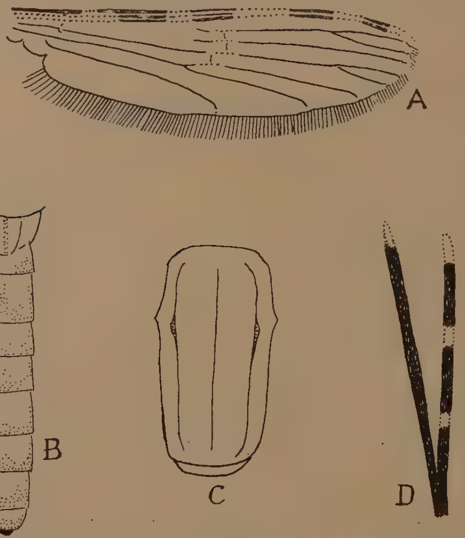

Fig. 1.—*Anopheles* (*Myzomyia*) *dthali wardi*, var. nov., adult: A. wing, B. dorsal aspect of abdomen, C. mesonotum to show three dark lines, D. proboscis and palp of female.

pale palpal bands of the female are well defined; the costal markings are more distinct; in some specimens the basal dark area of the costa is interrupted by a small pale spot; the basal fifth of the first vein is pale and there are some pale scales on other veins near the cross veins.

FEMALE.

Head: scales narrow and brownish. Vertical tuft, a few pale scales. Palps (fig. 1D), three narrow well defined pale bands, one of which is terminal. Pharyngeal armature resembles that of *A. dthali* but has 16–17 teeth instead of 11–12. *Thorax*: mesonotum (fig. 1C), integument pale, with three narrow, dark, longitudinal lines; brownish hairs only, no scales. *Legs*: femora, tibiae and tarsi all brown, with a few pale scales at apices of segments not amounting to bands. *Wings* (fig. 1A): length, 2·5 to 3·0 mm. Costa, four dark areas separated by well defined pale areas; first dark area occupies basal third of costa but may be divided about half-way by a small pale spot in some specimens. Subcosta and vein 1, pale in basal fifth with a dark area opposite distal portion of basal dark area on costa; other dark areas opposite those on costa. All other veins dark except at cross-veins, around which are some pale scales. Apex of wing, pale area from vein 2·1 to vein 4·1 which may be interrupted by a small dark fringe spot. Rest of fringe scales dark. *Abdomen* (fig. 1B): often pale and with a dark band at distal edge of segments expanded in the middle and at lateral edges, but sometimes all dark; hairs only, no scales.

MALE.

Relevant characters as for female. Palps dark with pale area at tips only. Hypopygium; harpago, with a stout bristle longer than the club and another bristle shorter than the club, between it and the club; phallosome, a little less than half the length of the coxite; leaflets, 8 or 9, some with serrated edges. (In one specimen the clubs are duplicated.)

LARVA.

A number of larvae and larval skins, which in the absence of " liaison specimens " are assumed to be those of this variety, are indistinguishable from those of *A. dthali*. Fronto clypeal markings (fig. 2A), three spots, middle one triangular with apex behind. Inner clypeal hairs separate, simple, long, slender. Outer clypeal hairs simple, slender, half length of inner hairs. Posterior clypeal hairs simple, reaching well beyond bases of inner hairs. Inner sutural hairs long, simple. Outer sutural hairs shorter, 2–4 branches, bases more anterior than those of inner suturals. Subantennal hairs, 30–40 branches. Apical antennal hairs as long as or slightly longer than the blades; split halfway into 3–5 branches. Shaft hair minute, simple, nearly half way along shaft. Palpal hair strong, many branches. Mentum (fig. 2B), an apical tooth and four on each side almost equally spaced. Shoulder hairs, inner and middle arising from separate tubercles; inner with about 16 branches, middle with about 14, outer, simple, sometimes bifid. Propleural hairs, 2 long, simple; 1 long, feathered. Mesopleural hairs, 2 long, simple. Metapleural hairs, 1 long, simple; 1 long, with 2 or 3 branches. Basal spines, I long, slender and sharp; II and III shorter than I, sharp. Thoracic palmate hairs present but poorly developed, about 14 leaflets. Abdominal palmate hairs on segment I, poorly developed, 8–12 leaflets; II, well developed, about 14 leaflets with shoulders and fine filaments half as long as leaflets; III–VII, well developed, about 17 leaflets with shoulders and fine filaments half to three-quarters length of filaments. Tergal plates; main, about as wide as alimentary tract, shallow back to front, hind margin straight or slightly concave, but that on VIII larger, with hind margin convex and medianly indented; accessories, three, arranged in a triangle, one median and a pair behind it. Pecten with about 14 teeth, 6 long and 8 short. Saddle hair long, slender, usually simple, sometimes bifid. Caudal hairs (fig. 2C); inner, 11–12 branches, 5 posterior ones hooked.

PUPA.

Apart from minor differences in the branching of certain hairs, the pupa differs from the type form in the extent of the paddle fringe. The outer edge of the paddle is bare in the basal half, whereas in *dthali* the fringe extends to the base (Christophers, 1933).

Spine A : III, minute, blunt ; IV–VII, successively longer ; VI, reaches half-way across next segment ; VIII, 12 branches. Hair B : III, delicate, 12 branches ; IV, 6 branches ; V–VII, 9–10 branches. Hair C : III, 9 branches ; IV, 5 branches ; V–VII, simple, long, reaching beyond succeeding segment to half-way across the next. Hair 4, III–VII, small, about 6 branches. Paddle hair hooked at tip. Accessory paddle hair about 5 branches. Paddle fringe, short spines on outer border from half-way, grading into hairs to opposite base of paddle hair.

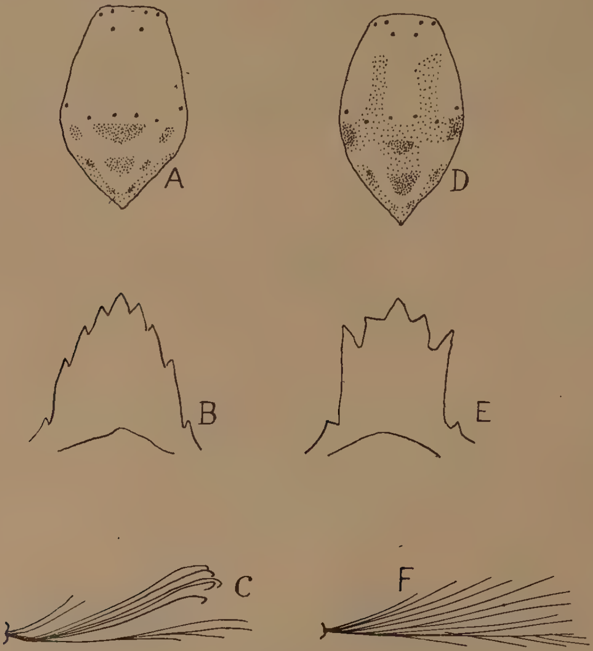

Fig. 2.—A-C. *Anopheles* (*Myzomyia*) *dthali wardi*, var. nov., larva ; D-F, *A.* (*M.*) *culicifacies* var. *adenensis*, Christ., larva for comparison. A and D, clypeus to show markings ; B and E, mentum ; C and F, upper caudal hairs.

Material examined. Adults bred, 82 ♀♀, 46 ♂♂ ; caught, 14 ♀♀. Larvae, several hundred. Pupae, 4.

Bionomics. *A. dthali* var. *wardi* appears to be common all over the island (fig. 3), and Capt. Ward records larvae from almost all types of water, *e.g.* running, standing, muddy, clear, brackish, fresh, shaded, open, seepage, pools, wells, at the edges of streams and lagoons, with or without alga (" flannel weed ", *Spirogyra* and *Chara*). He records also larvae of this Anopheline being taken alone on 67 occasions and with larvae of *adenensis* on 14 occasions.

Malaria. No dissections are recorded, but in Kallansiya, where this was the only Anopheline found, the malaria parasite rate was noted by Capt. Ward to be 78 per cent. in children up to twelve years of age.

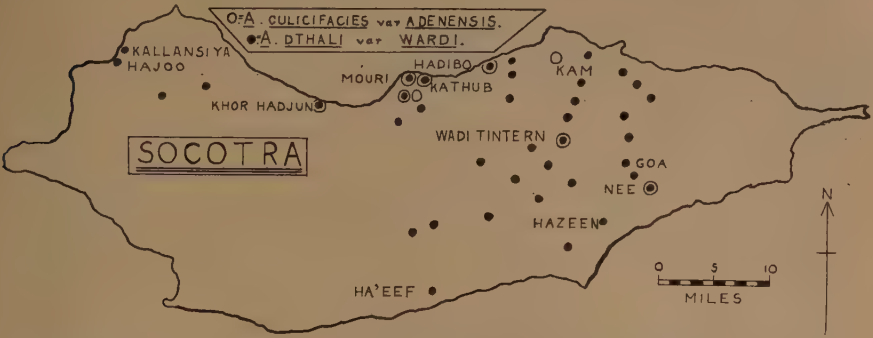

Fig. 3.—Sketch map of Socotra to show location of sites where Anopheline larvae were collected.

Anopheles (Myzomyia) culicifacies var. **adenensis,** Christ.

The character on which Christophers based his variety, *i.e.* the relative width of the pale areas on the costa, is not constant in the Socotra material ; in some specimens the pale areas are as narrow as or even narrower than in Indian specimens and in some distinctly wider. The variety is maintained, however, because the larvae show a constant characteristic head marking which consists of a dark Y-shaped pattern, the two arms of which extend towards the anterior border of the clypeus (fig. 2D). In larvae of the typical form the markings on the clypeus consist of two and sometimes three such spots behind the bases of the frontal hairs, these spots may or may not be connected with each other to form a dark band (Puri 1931).

Material examined. Adults bred, 21♀♀, 29♂♂ ; caught, 2♀♀, 6♂♂. Larvae, about 50.

Bionomics. *A. culicifacies* var. *adenensis* is commonest around Hadibo and Khatub in the northern coastal plain ; only at two points was it found outside this area (*i.e.* at Wadi Tintern and Nee, fig. 3). Larvae were found eleven times alone (eight times in wells), and fourteen times with larvae of *A. wardi* (three times in wells).

Malaria. No information.

The two Anophelines of Socotra may be distinguished as follows :—

Adults	*adenensis*	*wardi*
Wing	Pale scales at bases of forks and on veins 5 and 6	Veins 2 to 6 all dark except at cross veins
Head scales	Broad, black at sides, white in middle	Narrow and brownish
Male palps	3 pale bands on club	Pale at tip only
Larvae (fig. 2)		
Head markings	Y shaped pattern	3 spots behind frontal hairs
Mentum	Second lateral tooth much longer than others	Teeth equally spaced
Inner caudal hairs	None hooked	5 posterior ones hooked

CULICINES.

Aëdes (Stegomyia) granti, Theo.

The adult is easily recognised by the white lines on the proboscis and legs, the abdominal banding and the white line on the basal third of the costa.

The female was originally described by Theobald (1901), references were made to this species by Edwards (1912 & 1925) and a description of the female is included in Part III of "Mosquitoes of the Ethiopian Region" (Edwards 1941).

MALE.

Head: a median patch of white scales in the middle of which is a black line; a patch of white scales separated from the median patch on each side. White scales around eyes and bases of antennae. Proboscis with a dorsal and ventral line of white scales from base nearly to tip. Second segment of palpi almost completely white, dark distally; basal half of third segment white; fourth and fifth segments with a narrow basal white band incomplete above. *Thorax*: as female; "reddish brown; scutum with a narrow median line of narrow white scales extending the whole length and forking in front of scutellum; a narrow line of small but broad white scales commences on front margin on each side of median line, passes round margin to scutal angle where it turns inwards and continues back to scutellum; another line of small flat white scales commences in front of lateral lobe of scutellum, passes above wing root, and continues first along margin of scutum and then across posterior pronotal area. Scutellar scales white" (Edwards 1941). *Abdomen*: as female; "a narrow white basal band on middle part of each tergite which passes obliquely across sides of tergite to posterior corners" (Edwards 1941). *Legs*: on anterior surface a line of white scales extends the whole length of the femur and tibia and most of first tarsal segment; femora with a ventral white line. Fore- and mid-tarsi, second segment with a narrow basal white band, remainder brown. Hind-tarsi, second, third and fourth segments with basal half white and fifth segment entirely white. *Wings*: basal half of costa white, other veins dark. *Terminalia*: tergite, middle part convex with minute hair-like processes on and near border, lateral lobes with three or four long bristles; style long and slightly curved; terminal spine nearly straight, blunt and arising near apex of style; paraprocts, short, stout, with no basal arm; phallosome with numerous, closely approximated, blunt-ended teeth.

LARVA.

Head: length of antennal shaft hair about one-fifth the length of the shaft, arising beyond midway. Outer frontal hairs (A) bifid; inner frontal hairs (C) arising on a level with bases of antennae, long, reaching to end of antennae, simple; middle frontal hairs (B) arising near anterior margin of clypeus, simple or bifid, reaching as far as apex of antennae; anterior frontals (d) situated between bases of inner frontals, very short, with seven or eight branches; inner sutural hairs (e) arising level with front of eyes, short, simple; outer or trans-sutural (f) arising near eye, simple. Mentum, one median tooth and eleven teeth each side, closely arranged. *Thorax*: propleural hairs with no basal spine. The pleural tufts of meso- and meta-thorax have at their bases strong, bluntly pointed spines. *Abdomen* (fig. 4): comb consisting of nine to twelve spines, each with one main terminal denticle and a number of small basal ones. Siphon barrel-shaped, index about 2; subventral tuft with 4 or 5 branches arising at or more than two-thirds along siphon; pecten with eleven to twenty-two spines, each having a large main tooth and several small basal ones, no spines beyond tuft. Saddle, incomplete; saddle hair, 3–4 branches delicately barbed. Caudal setae simple, or one or two may be bifid. Papillae (gills); two long, two short, with parallel sides and rounded ends.

Material. 8 ♂♂, 7 ♀♀ bred from larvae collected from wells at Mouri; 3 larvae.

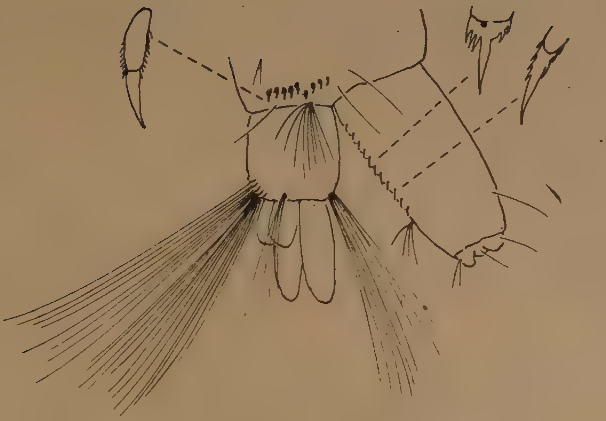

Fig. 4.—*Aëdes granti*, Theo., larva, terminal segments.

Aëdes (Stegomyia) vittatus, Big.

Collected as larvae from rock-holes at Hadibo, Kallansiya and Kathub; adults bred out.

The two species of *Aëdes* in Socotra are easily distinguished as follows :—

Adults	*granti*	*vittatus*
Proboscis	White line	Pale in middle
Tibiae	White line	White ring near middle
Pattern on mesonotum	Median straight line and lateral curved white lines	Six white spots
Abdominal white bands	Pass obliquely to posterior angles of tergites	Normal
Larvae		
Outer frontal hairs (A)	Bifid	4–8 branches
Anterior frontals (D)	7–8 branches	2–4 branches
Pecten spines	No spines beyond tuft	A spine beyond tuft
Ventral brush	No unpaired tufts	3 unpaired proximal tufts

The following species of *Culex* were collected as larvae and some adults were bred out.

Culex sitiens, Wied.

Lagoons, wells, crab-holes, streams, mangrove swamp; Mouri, Kathub, Khor Hadjun.

The larva regarded as that of *Culex sitiens* agrees with the Indian form as described by Barraud (1934) except in the length of the siphon. Barraud gives the siphon index as 2–2½, as also does Hopkins (1936) for the East African form; Borel (1930) for the Cochin China form gives the index as 3·5 or more while Lewis (1945) found it to be 4·5 to 5·9 averaging 5·1 in eight specimens from Port Sudan. In ten larvae in the Socotra collection the siphon index varied from 4·4 to 5·0, averaging 4·86.

Culex thalassius, Theo.

Pools ; Kathub.

Culex laticinctus, Edw.

Wells, rock-holes, seepages ; Mouri, Kathub and Hadibo.

Culex sinaiticus, Kirkp.

Lagoons, seepages, wells, pools ; Mouri, Kathub and Hadibo.

Culex fatigans, Wied.

Wells and earthenware jars ; Mouri, Kallansiya, Hadibo, Kathub and Hayoo.

KEY TO IDENTIFICATION OF ADULTS OF SPECIES OF *Culex* IN SOCOTRA.

1. Proboscis with well defined pale ring in middle..2

Proboscis without pale ring..3

2. Femora with scattered pale scales, base of upper fork cell distal to that of lower fork..*sitiens*, Wied.

Femora without scattered pale scales ; base of upper fork cell proximal to that of lower fork..*thalassius*, Theo.

3. Post spiracular and pre-alar scales present........................*sinaiticus*, Kirkp.

Post spiracular and pre-alar scales absent..4

4. Proboscis entirely blackish ; cross-veins not very wide apart ; lower mesepimeral bristles two or more..*laticinctus*, Edw.

Proboscis pale scaled beneath ; cross-veins wide apart ; lower mesepimeral bristle one only..*fatigans*, Wied.

KEY FOR IDENTIFICATION OF LARVAE OF SPECIES OF *Culex* IN SOCOTRA.

1. Comb of spines except for some scales in proximal part...........*sinaiticus*, Kirkp.

Comb wholly composed of scales...2

2. Sides of siphon markedly convex ; dorsal gills longer than saddle..................... *fatigans*, Wied.

Sides of siphon not markedly convex ; dorsal gills not longer than saddle.........3

3. Preclypeal spines thin ; pecten spines distinctly curved ; pecten extending beyond proximal tufts ; gills broadly lanceolate..........................*laticinctus*, Edw.

Preclypeal spines thick ; pecten spines not so curved ; pecten not extending beyond proximal tufts ; gills small and rounded....................................4

4. Comb with about 30 to 35 scales ; saddle hair as long as saddle......*sitiens*, Wied.

Comb with about 50 to 56 scales ; saddle hair about twice as long as saddle...... *thalassius*, Theo.

References.

BARRAUD, P. J. (1934). Fauna of British India, Fam. Culicidae. Tribes Megarhinini and Culicini. Taylor & Francis, London.

BECKER, Th. (1910). Dipteren aus Südarabien und von der Insel Sokotra.—Denkschr. Akad. Wiss. Wien, **71**, pp. 1–30.

BOREL, E. (1930). Les moustiques de la Cochinchine et du Sud-Annam. Masson et Cie, Paris.

CHRISTOPHERS, S. R. (1933). Fauna of British India. Fam. Culicidae. Tribe Anophelini. Taylor & Francis, London.

EDWARDS, F. W. (1912). A synopsis of the species of African Culicidae, other than *Anopheles*.—Bull. ent. Res., **3**, pp. 1–53.

——. (1925). Mosquito Notes.—V.—Bull. ent. Res., **15**, pp. 257–270.

——. (1941). Mosquitoes of the Ethiopian Region. III. Culicine adults and pupae. Brit. Mus. (Nat. Hist.), London.

EVANS, A. M. (1938). Mosquitoes of the Ethiopian Region. II. Anophelini. Brit. Mus. (Nat. Hist.), London.

FORBES, H. O. (1903). The natural history of Sokotra and Abd-el-Kuri. Liverpool Public Museums.

HOPKINS, G. H. E. (1936). Mosquitoes of the Ethiopian Region. I. Larval bionomics of mosquitoes and taxonomy of Culicine larvae. Brit. Mus. (Nat. Hist.), London.

——. (1942). Mosquitoes of the Ethiopian Region—notes and corrections.—Bull. ent. Res., **33**, pp. 175–179.

KIRKPATRICK, T. W. (1925). The mosquitoes of Egypt. Egypt. Govt. Anti-Malar. Comm., Cairo.

LEWIS, D. J. (1945). Observations on the distribution and taxonomy of Culicidae (Diptera) in the Sudan.—Trans. R. ent. Soc. Lond., **95**, pp. 1–24.

PURI, I. M. (1931). Larvae of Anopheline mosquitoes, with full description of those of the Indian species.—Indian med. Res. Mem., no. 21.

THEOBALD, F. V. (1901). A monograph of the Culicidae of the world. I. Brit. Mus. (Nat. Hist.), London.

AN ANNOTATED LIST OF INSECTS ASSOCIATED WITH GROUND-NUTS IN EAST AFRICA.

By W. F. Jepson, O.B.E., Ph.D.

Entomologist, Department of Agriculture, Tanganyika Territory.

It is now more than ten years since the publication of Harris' list of insects injurious to native food crops in Tanganyika (Harris 1937). It may be well to recall the view expressed in his introduction " . . . these crops . . . have achieved, within the limits of their present distribution, an equilibrium with their insect pests. Where there is no reasonable return for his labours, the native has ceased to grow a particular crop. This equilibrium is unstable, since pests increase in numbers at intervals owing to a variety of causes . . . ".

With the advent of the scheme for the mechanised production of ground-nuts in East Africa, it is desired to place at the disposal of those responsible for the protection of the crop against insect pests, the most up-to-date information as to the species which may be expected to appear sooner or later, wherever the ground-nut is cultivated. If the experience of this Department with the pests both of ground-nuts and of other common crops is any criterion, there is likely to be a restricted number of really major pests which may threaten to limit production over large areas unless energetic plant protection measures are adopted from the outset to prevent the build-up of large populations. In particular the seasonal arrangement of the other crops in the rotation will require careful watching to avoid the mass increase of pests common to a number of different crops. This list has been compiled from the records of this Department (Harris 1946, unpublished), and of those of Kenya and Uganda, assisted by Anderson's List of Insect Pests of East Africa, and by reviews of work carried out in the adjacent territory of Belgian Congo. A special watch has been kept during the past 18 months for the appearance of pests of both the growing and the stored crop, and collections have been received from the Area Manager at Kongwa (Mr. A. H. Noble) during 1947. It is a matter of interest that the first insect associated with the failure of a restricted area of plantation should be a new species of weevil belonging to the genus *Diaecoderus*.

Pests of the Standing Crop.

(Major pests are marked with an asterisk.)

Orthoptera.

Gryllidae.

Liogryllus morio, F. Tanganyika.

The common field cricket whose attacks on a variety of food crops become serious when growth is retarded for any reason. Found at Mlila, near Kongwa (Central Province) 1947 (*A. H. Noble*).

Acrididae.

Locusta migratoria migratorioides, R. & F. Uganda, Kenya, Tanganyika, Nyasaland, Belgian Congo.

The migratory locust, activity of which in the Lake Chad outbreak centre has recently been reported.

Schistocerca gregaria, Forsk. Uganda, Kenya, Tanganyika.

The Desert Locust, whose breeding foci in the Ethiopian area are being carefully watched by the East African Locust Directorate. Scattered escape swarms have been known to reach the Central Line Railway in the Mpapwa area. The outlook for the next few years is favourable, thanks to the energetic work of the Directorate.

Nomadacris septemfasciata, Serv. Uganda, Tanganyika, Nyasaland, Belgian Congo.

The Red Locust, with primary breeding focus in Lake Rukwa area. A campaign for the elimination of mass increase in the Malagarasi region near the Urambo ground-nut area (Western Province) is now in progress under the direction of Mr. F. B. Notley, Entomologist of this Department.

Chrotogonus rotundatus, Kirby.* Tanganyika.

A solitary grasshopper injurious to the young foliage of many ground crops. Mr. Harris expressed to the writer his view that this insect, together with related species and genera, and especially two species of *Eupropacris*, are liable to appear in large numbers in the area adjacent to the new Kongwa ground-nut region. Submitted 1947 from Sagara black soil area (*A. H. Noble*).

Isoptera.

TERMITIDAE.

Allodontermes morogorensis, Harris. Tanganyika.

An earth-nesting termite, which is constantly reported as destructive to young planted trees and to lawns during dry periods. It is probably the need for water that causes them to attack living tissue, for their normal habit is to scavenge any fragments of branches, roots, fruits, etc., which may fall on the soil. The rate of destruction of stover in Western Province maize fields by this or closely related species is phenomenal, and it is a matter for urgent attention to discover the relationship of termites in general to the structure of agricultural soils.

Thysanoptera.

There is a single record of the occurrence of thrips larvae in unopened leaves (Zimmermann 1913), and although nothing has been signalled in recent years, constant watch should be kept on young shoots during long dry periods.

Hemiptera.

PENTATOMIDAE.

Piezodorus pallescens, Germ. Uganda.

Cydnus sp. Tanganyika.

Closely related to the stink bug of wheat, young plants of which are often attacked at ground level. Sent from Mlila, near Kongwa UAC Plot 1947 (*A. H. Noble*).

JASSIDAE.

Empoasca sp. Tanganyika.

Empoasca dolichi, Paoli. Uganda.

Not known to transmit rosette, but of common occurrence and only once reported as injurious in Uganda.

TETTIGOMETRIDAE.

Hilda patruelis, Stål. Uganda, Tanganyika, Belgian Congo.

This leafhopper completely destroyed a small plot of Barberton ground-nuts planted out of season (June 1947) at Morogoro. Eggs are laid on the stems and young fruits, at or below ground level, hatching in 12 days (at 72°F.) to nymphs which suck the tender tissues causing yellowing and collapse of plants. A generation lasts about 6 weeks, and control was established in the third generation by an Encyrtid egg parasite. The nymphs and adults are farmed and protected by ants, which build earthen funnels around the bases of the stems. Alternative hosts of *Hilda* are the sunflower, and the ornamental flower *Coreopsis*. On sunflower, the nymphs and adults are found abundantly on the backs of maturing flowers. The species is not known to transmit rosette, which was nearly absent from the Morogoro trial plot.

ALEURODIDAE.

Bemisia sp. Uganda, Tanganyika.

Bemisia tabaci, Genn. Belgian Congo.

The whitefly, exonerated as a vector of rosette in Belgian Congo.

APHIDIDAE.

Aphis laburni, Kalt.* (*leguminosae*, auct.). Uganda, Tanganyika, Nyasaland, Belgian Congo.

This Aphid was shown by Storey to be the sole or principal vector of rosette. The disease was recorded by Professor Zimmermann in 1907 in German East Africa (Lindi district), but the difficulty of finding the Aphid in the field resulted in the long delay in identifying the vector. Figures from the Lake Province in the early days of the rediscovery of the disease in Tanganyika (1923) indicate that from 1 to 50 per cent. of the plants were affected according to area.

COCCIDAE.

Pseudococcus brevipes, Ckll.*

The common mealybug prevalent especially in badly drained plantings. Farmed by ants, the insects may cause blackening of the nuts.

Lepidoptera.

TORTRICIDAE.

Tortrix dinota, Meyr. Uganda.

PYRALIDAE.

Maruca testulalis, Geyer. Uganda. The lima bean pod borer.

GEOMETRIDAE.

Ascotis reciprocaria, Wlk. Uganda.

ARCTIIDAE.

Estigmene unipunctata, Hmpsn. Uganda.

Spilosoma investigatorum, Karsch. Uganda.

NOCTUIDAE.

Heliothis armigera, Hb. Northern Rhodesia.

Feeds on young shoots in the caterpillar stage. Not recorded in Tanganyika on ground-nuts but potentially dangerous wherever maize is included in a rotation.

Plusia acuta, Wlk. Uganda.

Plusia limbirena, Gn. Kenya.

Prodenia litura, F. Uganda.

Cotton leaf worms.

Laphygma exempta, Wlk.* and *L. exigua*, Hb.*

The East African army worms are liable to attack all grain and leguminous crops (Harris 1937). Control is dependent on early detection on wild grasses, especially star grass (*Cynodon dactylon*).

LIMACODIDAE.

Parasa vivida, Wlk. Tanganyika.

The stinging "slug" of coffee. A sporadic pest, usually rapidly controlled by parasites.

Coleoptera.

TENEBRIONIDAE.

Zophosis congesta, Sjöst. Tanganyika.

Zophosis sp. Tanganyika.

Dusky ground beetles sent from the Mlali ground-nut plots near Kongwa 1947 (*A. H. Noble*).

Catamerus revoili, Fairm. Tanganyika.

Emmallus major, Fairm. Tanganyika.

Macropoda nigrogemmata, Fairm. Tanganyika.

Peristeptus gestroi, Haag, var. Tanganyika.

Pogonobasis ornata, Sol. Tanganyika.

Rhytinota acuticollis, Fairm. Tanganyika.

Rhytinota gracillima, Ancey. Tanganyika.

All the above are unknown as pests, but were found on ground-nut plots at Kongwa 1947 (*A. H. Noble*).

MELOIDAE.

Coryna apicicornis, Guér.* Tanganyika.

Coryna lanuginosa, Gerst. Tanganyika.

Small black and yellow blister beetles numerous on flowers and young shoots of ground-nut in 1947 at Kingolwira Prison Farm (Morogoro District).

GALERUCIDAE.

Ergana bicolor, Jac. Tanganyika.

Associated with failure of young plantation at Kongwa, 1948 (*Bunting*).

Luperodes quaternus, Fairm. Uganda.

In certain areas, these leaf-feeding beetles will certainly cause trouble. Common legume-feeding species in Tanganyika are *Hallirhotius africana*, Jac., and *Ootheca bennigseni*, Ws.

CURCULIONIDAE.

Alcidodes (*Alcides*) *dentipes*, Ol.* Tanganyika.

The ground-nut stem girdler, of widespread and constant occurrence in native shambas.

Dereodus recticollis, Mshl. Nyasaland.

The larvae work at the base of young plants. Serious infestations may result if alternate host crops, tobacco, sunflower, cotton, or tobacco, are in the rotation.

Sphrigodes globulus, Mshl. Tanganyika.

Systates articollis, Mshl. Tanganyika.

These two species defoliate plants in all stages by skeletonising the leaves. Young plants are often attacked.

Diaecoderus sp. nov. Tanganyika.

Kongwa 1948 (*Bunting*), associated with the yellowing and failure of a young plantation.

PESTS OF THE STORED CROP.

(*a*) *Unshelled Ground-nuts.*

Lepidoptera.

PYRALIDAE.

Ephestia cautella, Wlk. Uganda, Tanganyika.

Moths emerged from a sample taken from a native seed store at Kigoma, Western Province in 1947, and from ground-nut seed stored for many months on the quay at Dar-es-Salaam by the Ground-nut Scheme authorities. In both cases the infestation was negligible compared with that found on closely adjacent sacks of shelled nuts.

(*b*) *Shelled Ground-nuts.*

Lepidoptera.

PYRALIDAE.

Ephestia cautella, Wlk.* Uganda, Tanganyika.

Universally present in native produce stored in commercial godowns; very heavy infestations have been seen.

Coleoptera.

TROGOSITIDAE.

Tenebroides mauritanicus, L.* Cosmopolitan.

The " Cadelle " is very destructive to ground-nuts in bag stores, possibly following upon primary infestations by *Ephestia*, upon whose larvae it is predacious.

TENEBRIONIDAE.

Tribolium castaneum, Hbst. Cosmopolitan.

A secondary pest indicative of neglected primary infestations by other species.

References.

HARRIS, W. V. (1937).—Bull. ent. Res., **28**, pp. 483–488.

MOSQUITOS AND MALARIA IN THE KABAW AND KALE VALLEYS, BURMA.*

By T. T. MACAN, M.A., Ph.D., F.R.E.S., Major, R.A.M.C.
No. 1 Malaria Field Laboratory, and Laboratory of the Freshwater Biological Association, Wray Castle, Ambleside, Westmorland.

(Plate XXI.)

CONTENTS.

	PAGE
General description of the valleys	237
History of observations and technique	240
Distribution of malaria	242
The Anopheline population	243
The vectors	251
Notes on resting places	256
Rate of ovarian development	258
Further observations at night	258
Notes on time of activity	259
Attraction to light	262
Systematic notes	262
Acknowledgements	266
Summary	266
References	268

General Description of the Valleys.

The Kabaw and Kale valleys are often included together under the name Kabaw Valley, though strictly this name only applies to the more northerly of the two; they are situated on the eastern or Burmese side of the high range of mountains which lies between the plains of India and the plains of Burma (fig. 1). They extend roughly from latitude 23° to 25° north just within the frontiers of Burma and run in a north and south direction separated from the valley of the Chindwin by a range of hills that rises in places to over 3,000 feet above sea level. The valleys themselves are about 500 feet above sea level. The total length of the two together is nearly 250 miles, but the area covered during the course of the work to be described comprised only a stretch of about 100 miles along the main road from Tamu to Kalemyo, which is near the confluence of the two rivers draining the Kale Valley, in the latitude of Kalewa (fig. 1). The width varies from about 4 to about 10 miles. The watershed dividing the two valleys scarcely rises 100 feet above the general level of the valley bottoms and it is hardly perceptible when driving along the road although from the air it is more obvious. A peculiarity of both valleys is that the rivers drain from the north and the south, meet near their centres and then flow through deep and narrow gorges to the Chindwin.

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* Permission to publish these results has been granted by the Director-General of the Army Medical Services in letter A.D.H./DO/47 dated 31.i.47 (AMD5). The War Office does not accept any responsibility for statements made or conclusions drawn.

The valley bottoms are flat and almost the whole of the Kabaw Valley is covered by trees, which do not, however, grow very close together so that from the air individual trees can be made out and the tree tops do not present a continuous canopy (Plate XXI, fig. 1). Beneath the trees there is a thick growth of shrubs and herbs, though much of the area studied had been camped on at one time or another so that the undergrowth had been cleared and the vegetation at the time of survey was young trees and quite small herbs. Population is very sparse in the Kabaw Valley and clearings are few and small; in the Kale Valley population is denser and the whole of the centre of the valley has been cleared. Rice is the main crop grown.

One of the chief land routes between India and Burma runs through the two valleys. It comes from Imphal, winding over the mountains and enters the Kabaw Valley at Tamu, whence it runs south, crossing the low watershed into the Kale Valley and finally, following the river draining this valley through its gorge, to the Chindwin at Kalewa. From Tamu roads also lead north and east and both these roads were surveyed for a few miles, but the rest of the work was confined to the neighbourhood of the through road.

The rocks of the mountains flanking the valley are metamorphic with a capping of red loam. There is a variable depth of alluvial soil in the valleys and, at the few points at which the substratum was exposed, it was observed to be an apparently impervious gravel conglomerate. Many streams flow across the valleys to join the central rivers. Where the top soil is thick, they flow in deep gorges and sometimes disappear underground but where the top soil is thin they flow through broad marshy areas where biological conditions approximate more closely to those of a pond than to those of a stream. Among the more conspicuous features of a somewhat featureless landscape are long marshy areas known as "luwins" that run through the forest, following a sinuous course, and catch the eye at all times of year because they are without trees. The breadth is up to 200 yards, and the length may be half a mile or more. In the rainy season they contain about a foot of water and there is a good deal of emergent and other aquatic vegetation, with some flow down the centre. No exact observations were made but probably most of them dry out during that period of the year when no rain falls. They have attracted much attention and speculation and they have a reputation for being malarious though there appeared to be no reason why they should be more malarious than any other parts of the valley.

Various theories have been put forward to account for their existence and, as some of these seem to have little foundation in fact, it is proposed here to propound what seems to be the most plausible explanation which was first put forward by Lt.-Col. G. I. Watson. It has already been pointed out that the valleys do not drain out of the lower end in the usual way but through a gorge in the side. This gorge has obviously been cut quite recently, geologically speaking, and probably at present it is still being deepened. It seems reasonable to postulate that the deepening of the gorge is not a process which takes place gradually but that appreciable drops in level take place suddenly when some barrier, which, being harder than the neighbouring rocks has eroded less rapidly, suddenly gives way under the strain of an unusually heavy flood. Such an event would lead to a drop in the level of the main river in the valley itself.

Some of the side streams are large and have built up considerable deltas with many distributaries where they enter the valley. After a drop in the general water level such as that described in the preceding paragraph, a stream would start to erode its delta and would continue until a large sector in the centre of the delta had been removed. When it had eaten down to a level at which flow was once more sluggish, it would start to build up a second delta. The processes of delta formation and erosion might alternate several times. Each time erosion started the distributaries would be left isolated but their beds might be waterlogged during the rains and it is suggested that this is the way in which the luwins have been formed. It is also to be

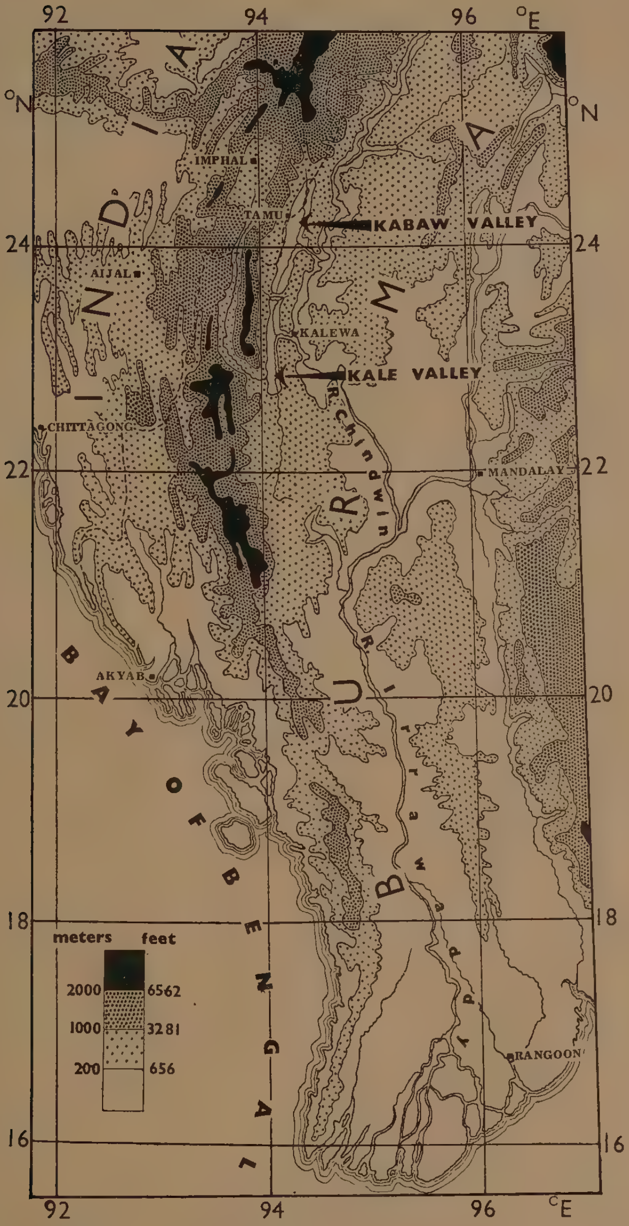
92
94
96
°E
°N
INDIA
BURMA
IMPHAL
TAMU
KABAW VALLEY
AIJAL
KALEWA
KALE VALLEY
R. Chindwin
CHITTAGONG
MANDALAY
AKYAB
BAY OF BENGAL
R. Irrawaddy
RANGOON
24
22
20
18
16
meters
feet
2000
6562
1000
3281
200
656

Fig. 1.—Sketch map to show the position of the Kabaw and Kale Valleys.

expected that, where the edge of a later delta abuts against the eroded cliff face of the delta preceding it, there will be a channel in which water will accumulate. The structure of the country round Tamu, where the Lokchao river flows into the valley and where luwins are particularly numerous and well developed, supports this hypothesis. The river itself flows between high banks and does not overflow in the time of flood, so it would appear to be in an eroding stage. Flat land lies beyond the river banks and then there is an escarpment on which the village of Tamu stands. A luwin flows along the foot of this escarpment for part of its length. From the top of the escarpment the land slopes away very gently and it is traversed by several luwins. About a mile from Tamu there is another escarpment with a large luwin flowing along its foot. An opportunity occurred to fly over this area and it was possible to observe on the other side of the river the two corresponding escarpments which the theory demands. Further, it will be obvious that, if the explanation advanced be the correct one, a luwin at the foot of an escarpment should be at a lower level than a luwin on the land beyond the escarpment. Through the kindness of Major Pettman, R.E., commanding 924 Indian Works Section, a line between two such luwins was surveyed and the levels were found to be as expected.

It is suggested, therefore, that the luwins are the channels of old distributaries fanning out over a delta that is now above the level of the stream which originally built it up. In addition to the luwins there are similar mosquito breeding places on a much smaller scale. During the rains, water stands in natural hollows and depressions and also in old camp sites, in slit trenches and ruts. It was difficult to form a true picture and decide whether or not these artificial breeding places added significantly to the total available, as progress through the uncleared jungle was slow and laborious and could not be achieved without the aid of a big knife, and it was never possible to view more than a few yards of the land round about. Other breeding places during the rains were the rice fields, both those in use and those which had been left derelict. Most of these abandoned rice fields were on flat land and rain kept them filled with water throughout the period of the rains ; a few, however, were on sloping ground and were stream-fed in times of cultivation and, in the absence of any regulation, they drained rapidly and rainwater did not accumulate in them.

When the rains ceased these various stagnant breeding places rapidly disappeared and many of the smaller streams gradually dried up as well.

No exact information about the climate is apparently available. The south-west monsoon brought rain in May and there was heavy rainfall throughout the months of June, July, August and September. The last heavy rain in 1944 fell on 4th October and for the rest of the year there was little rain. Temperature was fairly high throughout the year though noticeably lower during December and January.

The inhabitants lived in houses built to the typical Burmese pattern, some 6 or 7 feet off the ground and cattle were tethered at night between the posts supporting the house. The living floor consisted of a veranda, a large room behind it with the whole front open and one or more small sleeping rooms, completely walled and provided with doors. The better houses were built of planks, fitting edge to edge so that the bedrooms were dark but the walls of the poorer houses were of bamboo matting which let in a lot of air and light. Many of the villages had been destroyed during the various campaigns, and the inhabitants had moved to temporary quarters, so that most of the houses, where mosquito collections were made, were of the poorer type.

History of Observations and Technique.

Prior to the war no observations had been made in the valleys under discussion and, in fact, knowledge about mosquitos and malaria in the whole of Burma was scanty. In 1942 part of the allied forces retreated through the Kabaw and Kale

Valleys. Lt.-Col. Jarvie was with them as Malariologist and he was able to find out the spleen rate at many of the villages. These records are of special value as at that time the population had not been disturbed by the war and there was no danger, as there was subsequently, that the results at any one place had been affected either by an influx of refugees or by a temporary flight of the autochthonous population into the forest while the battle passed by.

In 1943 no records were made as far as is known. During the first four months of 1944, Nos. 20, 55 and 69 Anti Malaria Units operated around Tamu and their records have been available, but they had to withdraw in April on account of the Japanese advance on Imphal. In August 1944, 11th (East African) Division started to advance from Tamu, and at the end of the month a party from No. 1 Malaria Field Laboratory was sent to the Kabaw Valley to make a malaria survey. Thereafter sections of this Unit, charged with various missions, were in the valley on and off until May 1945 ; the officer in charge on each occasion was the present author, who was entomologist to the Unit. A field trial with DDT sprayed from the air was carried out in October 1944, and the preliminary catches before spraying and the results from control stations during the course of the experiment provided material for the present discussion. The end of November and most of December 1944 was to have been spent observing the effects of an operational air spray with DDT in the Kale Valley, but, as the programme was changed at the last minute and the stations which had been kept under preliminary observation were not sprayed, no information about the effect of the spray was obtained. But the loss on this count was a gain to epidemiological studies. In February 1945, two sections travelled through the valley and were able to revisit some of the catching stations of the previous year. A week, early in May 1945, was devoted to catching and dissecting mosquitos, the intention being to carry out experiments with DDT sprayed from the ground. The section, however, was recalled on the day after the spraying had actually begun.

The author was assisted by Squadron Leader Roberts, R.A.F., attached for instruction in September, by Capt. (later Major) D. G. R. Fox in October, by Major (later Lt.-Col.) G. I. Watson in February and by Capt. (later Major) J. H. Gerber and Capt. R. Shanks in May.

Valuable co-operation was received from 21st (East African) Mobile Malaria Section commanded by Major G. L. Broderick, R.A.M.C. In October he detached a sub-section under Staff Sgt. Shepherd to assist with the DDT airspray observations. Lt. Topping of the same Unit organised a large series of larval collections in the latter half of October and in November and a smaller series later in the year, and has kindly placed the results at my disposal.

Thus observations have been made in almost every month. The results are somewhat uneven; only one month was devoted entirely to surveying, and different units of different types contributed the data, but it is nevertheless possible to reconstruct a reasonably good picture of the mosquito population and its relation to malaria at different times of year in the valleys.

Nearly all the adult collections made by No. 1 Malaria Field Laboratory were made at night, either in camps or in villages. The friendliest co-operation was extended by the inhabitants in both types of catching place. The villagers were all Buddhists and appeared to have no objection to collectors entering and searching their houses at all times of the night. The camps were all sufficiently far behind the fighting for there to be no veto on flashing a torch during the hours of darkness. Many of the mosquitos were, of course, unfed and were quick to move if scared and a sucking-tube was found to be the only practicable method of securing specimens. Nearly all were taken while resting near their prey either before or after feeding ; only on one occasion was an appreciable number of specimens taken while they were actually biting (see p. 258).

Two different regimes were employed for night catching according to the purpose for which specimens were required. When dissection was the object one person collected all night, usually hourly, though often half-hourly if mosquitos were coming in fast and the camp or village was so small that a complete round could be made in half an hour. When a survey was being carried out and the party was on the road, a round was made at about 22.00, midnight, 02.00 and 04.00 hours, each member of the party taking one spell; if there were only three, the same person took the first and last round.

Larvae were usually collected with a ladle and the technique calls for no comment. The difficulty of locating breeding places may be referred to again here. Sometimes near stations where a big adult catch was made, search for the breeding places was unsuccessful; and it was not possible to assess the chances of a good adult catch by looking at the countryside around. In fact, the headquarters for the air spray experiment at Tamu was chosen because it appeared likely to be a particularly good place for mosquitos; in practice it yielded the poorest catches of any station in the valley.

Distribution of Malaria.

Spleen rates have been recorded at the places shown in Table I in which they are arranged in order from north to south. The month and the year when the spleen rate was taken is given where it is known; all the spleen rates in 1944 and 1945 were

TABLE I.

Spleen rates in the Kabaw and Kale Valleys.

Valley	Place	Grid reference	Spleen rate	Date	Remarks
Kabaw	Myothit	SF 001280	100%		
	Mintha	RK 910180	96%		
	Kuntuang	RK 809051	86%		
	Tamu	RP 790920	85%		
	Tamu		60%	Mar., 1942	
	Tamu	RP 815923	50/68= 73%	Aug., 1944	New village
	Hesin	RP 823898	85%		
	Nakala	RP 781883	95%		
	Pantha	RP 768798	80%		
	Indaing	RP 785800	92%		
	Witok	RP 713653	83%		
			50/60= 83%	Sept., 1944	
	Sunle	RP 717561	67%		
	Minthami	RP 7555	69%		
	Zibyugon	RP 715520	75%		
			12/16= 75%	May, 1945	
	Malu	RP 693513	69%		
			15/18= 83%	May, 1945	New and old village
	Htinzin	RP 655495	62%	Mar., 1942	
		RP 6448	25/30= 83%	May, 1945	New village
	Teinkaya	RP 706433	73%		
Kale	Yazagyo	RP 5604	70%	Mar., 1942	
	Okkam	RU 574997	26/28= 93%	Nov., 1944	
	Kyetpanel	RU 566980	64/70= 91%	Nov., 1944	
	Kangyi	RU 563935	65%	Mar., 1942	
			93/94= 99%	Nov., 1944	
	Inbaung	RU 565817	57/72= 79%	Nov., 1944	
	Indainggyi	RU 6174	43%	Mar., 1942	
	Nanzalu	RU 541692	41/44= 93%	Dec., 1944	
		RU 538687	21/21=100%	Dec., 1944	(Refugees from Kalemyo)
	Kalemyo	RU 5667	66%	Mar., 1942	

made by the present author: the rest were obtained from records at 14th Army Headquarters. It is believed that many of the spleen rates shown without a date were obtained in 1942 by Lt.-Col. Jarvie.

The malaria risk is uniformly high throughout the length and breadth of the valleys. At all the villages visited mosquito nets were seen, and though many of them were in a tattered condition, it is possible that without them spleen rates would have been higher.

I was fortunate enough to meet Dr. Singh who was in charge of the hospital at Tamu in peacetime. His opinion was that malaria is transmitted throughout the year but that it is at its worst from June to November. Lt.-Col. Jarvie, who was able to consult the hospital records which were destroyed when Tamu village was burnt down in 1944, reported that there is much malaria between May and December inclusive.

The Anopheline Population.

The following is a complete list of species taken. The names are those given by Christophers (1933): a synonym is only shown where it may be more familiar than the valid name.

A. aitkeni, James 1903.
A. hyrcanus var. *nigerrimus*, Giles 1900.
A. barbirostris, Van der Wulp 1884.
A. kochi, Dönitz 1901.
A. leucosphyrus, Dönitz 1901.
A. tessellatus, Theobald 1901.
A. culicifacies, Giles 1901.
A. minimus, Theobald 1901.
A. aconitus, Dönitz 1902.
A. jeyporiensis, James 1902.
A. jeyporiensis var. *candidiensis*, Koidzumi 1924.
A. subpictus, Grassi 1899 (*rossii*, Giles 1899).
A. vagus, Dönitz 1902.
A. stephensi, Liston 1901.
A. maculatus, Theobald 1901.
A. splendidus, Koidzumi 1920 (*maculipalpis* var. *indiensis*, Theobald 1903).
A. annularis, Van der Wulp 1884 (*fuliginosus*, Giles 1900).
A. philippinensis, Ludlow 1902.

So many forms of *A. jeyporiensis*, intermediate between the type and the variety, were found that it was decided to record all specimens as *A. jeyporiensis*. Other systematic difficulties are dealt with on pp. 262–265.

The rains brought by the south-west monsoon are probably by far the most important of all the seasonal factors affecting the Anopheline population. The only observations during the rains were those made by No. 1 Malaria Field Laboratory between 26th August and 21st September and so these can conveniently be taken as a starting point. The work, as already noted, was carried out in the neighbourhood of Tamu and along each of the roads leading from it.

Larval collections were made in 25 breeding places and the results are shown in Table II.

TABLE II.

Larval collections in the Tamu area, 26th Aug.–21st Sept., 1944.

Species ...	*minimus*	*philippinensis*	*leucosphyrus*	*jeyporiensis*	*annularis*	*hyrcanus*	*aconitus*	*barbirostris*	*kochi*	*culicifacies*	*maculatus*	*vagus*	*subpictus*
Total number of larvae caught	2	33	18	7	15	144	6	58	10	8	6	19	2
Number of breeding places ...	1	8	4	5	7	19	2	15	2	2	3	3	2

A. hyrcanus, *A. philippinensis* and *A. annularis* were found breeding in derelict rice fields. In luwins the same three species occurred, *A. hyrcanus* being by far the most numerous, and in addition *A. leucosphyrus*, *A. jeyporiensis*, *A. aconitus* and *A. barbirostris*. *A. jeyporiensis* and *A. aconitus* were easily overlooked as they tended to occur where the emergent vegetation was thickest, sometimes so thick that the existence of a habitat for an aquatic organism was not immediately apparent. If, however, an attempt was made to walk through such places the feet became surrounded by a pool at each step and, in the pools so formed, larvae of these two species could be caught. *A. barbirostris* was also found in small forest pools. Some *A. leucosphyrus* were found in an old slit trench and Lieut. Topping of 21st East African Mobile Malaria Section found several slit trenches containing many larvae of this species. It was also taken in abandoned ammunition boxes and in a pool of water on a tarpaulin left in the forest.

Seven adult catching stations were visited and from one collections were made on five separate nights. The results are set out in detail in Table III.

TABLE III.

Adult collections in the Tamu area ; 12 night catches at 7 stations, 29th Aug.–21st Sept., 1944.

Place	Type and location	*minimus*	*philippinensis*	*leucosphyrus*	*jeyporienis*	*annularis*	*hyrcanus*	*aconitus*	*barbirostris*	*kochi*	*tessellatus*	*culicifacies*	*maculatus*	*vagus*	Total
Hesin	Village in clearing ...	2	31	—	2	8	—	—	—	—	—	4	1	—	48
Hesin	do. do.	1	4	—	—	2	—	—	—	—	—	—	—	—	7
Witok	Camp in clearing ...	6	15	—	4	14	34	—	7	1	—	—	—	—	81
Sunle	do. do.	22	5	—	—	15	22	—	—	—	—	—	—	1	65
Tamu	Deserted village at edge of forest ...	69	46	18	14	10	—	14	2	—	—	—	—	1	174
Moreh Bridge*	Camp in forest	52	12	15	7	3	1	2	1	1	—	—	—	(1)	468
Ya-nan	do. do.	3	3	17	—	1	2	—	—	—	1	—	—	—	27
Mile 9½	do. do.	4	12	17	9	72	3	—	2	—	—	—	2	—	121
	Total ...	366	177	128	63	136	66	25	14	5	1	4	3	3	991

* The figures for Moreh Bridge show the average number of each species caught in five nights but the figures in both the vertical and horizontal columns labelled 'total' include every mosquito taken.

The totals for each station are not all comparable. At Mile 9½ about 100 specimens were collected in one hour and the total catch was estimated to be about 300. It had to be transported next day 10 miles on foot in pouring rain along a road crossed by flooded streams, one of which had swept its bridge away, and the totals shown are the numbers of each species which were in a fit condition the next day for identification and dissection. The rest of the totals represent the whole night's catch but, whereas Witok, Sunle and Ya-nan were visited on the march and collecting was restricted as described above, collections from Tamu, Hesin and Moreh Bridge represent a longer period of activity during hours of darkness.

Attention may be drawn to the following points. All the catches were fairly large except that made on the occasion of the second visit to Hesin. *A. minimus* was taken in largest numbers but this is due to the fact that it was particularly abundant at Moreh Bridge where five collections were made ; at four of the seven stations it was scarce and the conclusion was that during the rains it is only locally abundant ; the larval collections bear this out as do the adult collections made in October (Table VII). *A. leucosphyrus* was not recorded at any of the stations in a clearing though one was barely one hundred yards from the edge of the forest, but in the forest, though not numerous, it occurred at all four stations. Other common species were *philippinensis*, *annularis*, and *jeyporiensis*. *A. hyrcanus* and *A. barbirostris* were encountered more frequently as larvae than as adults which suggests that they do not readily attack man. It is not possible, as might be supposed, to state definitely that cattle, as an alternative source of food, were present in villages and absent in camps, for stray cattle, presumably from some village which had been destroyed, were often found in unexpected places.

The next series of observations was also made round Tamu and had as its primary objective the assessment of the effect of DDT sprayed from the air. Intensive collecting to obtain information about the adult population before spraying started was carried out between 27/28 September and 3/4 October inclusive at four forest stations two of which, Tamu old village and Moreh Bridge, had been visited during the September survey. Several stations could be worked each night as the party was now larger, and in the seven nights twelve collections were made ; the results are presented in Table IV.

TABLE IV.

Adult catches in the Tamu area ; 12 night catches at 4 stations, 27th Sept.–4th Oct., 1944.

minimus	*philippinensis*	*leucosphyrus*	*jeyporiensis*	*annularis*	*hyrcanus*	*aconitus*	*barbirostris*	*kochi*	*tessellatus*	*culicifacies*	*maculatus*	*vagus*	*subpictus*
103	96	110	63	36	61	30	29	6	12	—	1	8	5

Compared with twelve catches made in September the total is smaller. This is due to a consistently poor catch from one station which was visited on five nights. Detailed comparison shows that *leucosphyrus* is relatively more and *minimus* less abundant but it is doubtful if these differences are of more than local significance. Larval catches during the same short period from ten places, most of which were in the clearing in and around Tamu, were made by Staff Sgt. Shepherd's African collectors and the results are shown in Table V.

TABLE V.

Larval catches in the Tamu area, 28th Sept.–4th Oct., 1944.

Species	*minimus*	*philippinensis*	*leucosphyrus*	*jeyporiensis*	*annularis*	*hyrcanus*	*aconitus*	*barbirostris*	*kochi*	*maculatus*	*vagus*
Total number of larvae caught ...	4	28	13	2	17	31	3	34	28	2	79
Number of breeding places	1	4	3	2	2	5	1	3	3	1	8

Detailed comparison with the September catches shown in Table II is not admissible since the former were rather haphazard collections over a large area and the latter a detailed examination of a small area. The general picture is similar.

All three breeding places of *A. leucosphyrus* were puddles on or beside roads and one was sunlit. The richest catch came from a luwin which yielded all of the eleven species recorded except *leucosphyrus* and *vagus*, the most abundant species being *hyrcanus*, *annularis* and *philippinensis*. A shell crater also contained many larvae although it was muddy and there was no cover except that provided by grass growing at the edge and trailing in the water ; the catch was *barbirostris* 26, *vagus* 26, *philippinensis* 13, *hyrcanus* 9.

The Africans worked the same area after it had been sprayed from the air. Larvae fairly soon reappeared and the catches made during the course of work, which continued till 28th October, are shown in Table VI.

TABLE VI.

Larval collections in the Tamu area after air spray with DDT, 6th–28th Oct., 1944.

Species	*minimus*	*philippinensis*	*leucosphyrus*	*jeyporiensis*	*annularis*	*hyrcanus*	*barbirostris*	*kochi*	*maculatus*	*vagus*
Total number of larvae caught	16	28	—	7	5	245	87	23	41	41
Number of breeding places	3	4	—	2	2	10	4	1	4	6

A. hyrcanus was definitely more abundant and occurred both in luwins and shell craters ; a visit to the rice fields outside the area studied showed that it was breeding in large numbers there also. The numbers of *A. maculatus* too are significantly larger and, although most of the larvae were found in running water, where they were to be expected, a few were also found in two of the four shell craters visited. *A. leucosphyrus* on the other hand disappeared, all three breeding places where it had been found earlier having dried up. The relative abundance of the remaining species is not greatly changed as far as can be made out from the rather meagre data available.

During the spray experiments adult collections were made from control stations outside the sprayed area. Eight nights were spent in two stations which lay outside the forest but which were connected to it by a zone of thick bushes, and one night was spent at each of four stations in the forest ; the catches are shown in Table VII.

TABLE VII.

Adult collections in the Tamu area ; 12 night catches from 6 stations, 29th Sept.—20th Oct., 1944.

minimus	*philippinensis*	*leucosphyrus*	*jeyporiensis*	*annularis*	*hyrcanus*	*aconitus*	*barbirostris*	*kochi*	*tessellatus*	*maculatus*	*vagus*	*splendidus*
75	250	28	96	105	450	12	18	8	3	1	2	1

Comparison with the figures for the September survey (Table III) must be guarded since the area was not identical, and a high proportion of the October catches were not made actually in forest. The number of *A. hyrcanus* probably represents a genuine increase since more larvae were being found at this time and this conclusion probably applies also to *A. philippinensis.* Similarly, the lower number of *A. leucosphyrus* in October probably indicates a decrease since larval breeding places had been found to be drying up. The decrease in the total of *A. minimus*, on the contrary, is probably due to the fact that it is only locally abundant at this time and the six October stations happened to be in places where it was scarce. The figures for the remaining species warrant no conclusion.

The total adult catches in the stations that were sprayed were still much below their pre-spray level when observations were concluded towards the end of October and for this reason they have been omitted from this account. No further observations were made in the Tamu area, and so a brief summary may be interpolated here.

The mosquito population in October, after the rains have ceased, shows no great change when compared with that of September ; the main differences are an increase in the numbers of *A. hyrcanus* and *A. philippinensis*, and a decrease in the numbers of *A. leucosphyrus.*

The next set of records to be noted is a series of larval collections made by 21st East African Mobile Malaria Section at Yazagyo. These started on 13th October, and thus coincided in time with the Tamu collections for about a fortnight, but they continued for longer, and included nearly the whole of the month of November. The results were presented by weeks, but it was noticeable that the catches in the first two weeks were much lower than in subsequent weeks, and it seems likely that in these first weeks the Unit was getting into its stride after the move, and the figures have, therefore, little comparative value. Further, figures from a unit which is also destroying mosquitos cannot be taken as a reliable guide to natural changes in population and accordingly in Table VIII the catches are shown as the total for the whole period.

TABLE VIII.

Larval collections in the Yazagyo area, 13th Oct.–26th Nov., 1945.

Species	*minimus*	*philippinensis*	*leucosphyrus*	*jeyporiensis*	*annularis*	*hyrcanus*	*barbirostris*	*culicifacies*	*maculatus*	*vagus*	*subpictus*	*aitkeni*
Total number of larvae caught	96	27	176	15	6	143	1,017	2	124	111	9	8
Number of breeding places	32	9	29	10	4	45	175	2	24	29	4	3

Yazagyo was in the forest and is, therefore, comparable with Tamu, but the two places are some 50 miles apart. There are differences in the fauna, notably a smaller proportion of *A. hyrcanus* and *A. philippinensis* and a higher proportion of *A. leucosphyrus*, but these differences are not greater than are to be expected from two different, albeit comparable, places. The catches at Tamu in October and the catches at Yazagyo, which extend to the end of November, cannot, therefore, be used to deduce much about seasonal trends in population. There is, however, some indication from the weekly figures that both *A. hyrcanus* and *A. philippinensis* were decreasing in abundance throughout the six weeks during which collections were being made at

Yazagyo. The persistence of *A. leucosphyrus* at Yazagyo, in contrast to its rapid decrease in numbers at Tamu soon after the rains ceased, is interesting ; it was found breeding in stagnant backwaters of large streams, so evidently it may persist well into the dry period where suitable conditions exist locally.

The 21st Mobile Malaria Section moved further south during November and between 24th November and 7th December made the following collection in three streams and a swamp near Inbaung (Table IX).

TABLE IX.

Larval collections in the Inbaung area, 24th Nov.–7th Dec., 1944.

Species	*minimus*	*philippinensis*	*leucosphyrus*	*jeyporiensis*	*annularis*	*hyrcanus*	*aconitus*	*barbirostris*	*maculatus*	*vagus*
Total number of larvae caught	25	3	1	27	7	19	10	86	6	2
Number of breeding places	3	1	1	1	1	2	3	4	2	1

A. minimus is now a more important component of the larval population than at any previous date. A good catch of *A. jeyporiensis* indicates that the scarcity of this species at Yazagyo was due to a local lack of suitable breeding places rather than a seasonal phenomenon. By this time, however, two months after the rains had ceased, stagnant water breeding places were much reduced in numbers and extent, and the population of mosquitos breeding in them was much lower, considered in relation to the whole area. The swamp, from which the collections in Table IX were made, was selected after some searching for a suitable place for further observations on the effect of spraying DDT from the air.

The 21st Mobile Malaria Section made no adult catches and there are no such records for the greater part of November.

Between 25th November and 20th December three villages in the Kale Valley were visited by a Mobile Section of No. 1 Malaria Field Laboratory. The results of five night collections in these three villages were *A. minimus* 363, *A. annularis* 5, *A. aconitus* 4, *A. barbirostris* 3, *A. kochi* 1, and *A. vagus* 1.

The contrast between this catch, in which *A. minimus* is the only abundant species, and the varied catches earlier in the year is striking, but conditions, it must be noted, were not the same, for the Kale Valley villages stood in a big clearing extending right across the valley, while in the Kabaw Valley, where all the earlier catches were made, the catching stations were in or near forest. Unfortunately it was not possible to visit the Kale Valley at any other season to obtain comparative catches but, when the larval population and the adult catches made in the Kabaw Valley in the following year are taken into consideration, there seems good reason to assert that the absence of *A. philippinensis*, *hyrcanus* and *jeyporiensis* from the Kale Valley at the end of November and in December is due to the fact that the season for those species is past. *A. leucosphyrus*, on the other hand, was not to be expected in the Kale Valley villages as it is a forest species.

The villages stood beside a river or large stream of which considerable stretches were stony. Among the stones along the edge larvae of *A. maculatus* were found, and the total population of this species must have been very large ; its complete absence from night catches made in the neighbourhood of man indicates that in this part of its range it has no importance as a vector of disease. Larvae of *A. minimus* were more restricted and occurred along the river's edge only in backwaters where there was an earthy bank and some vegetation, or where seepage occurred. Larvae of *A. stephensi* were also found in the streams.

In late February of the following year two parties of No. 1 Malaria Field Laboratory passed through the Kabaw and Kale Valleys. A night was spent in the luwin area near Tamu on a site where, in the previous October, 21 *hyrcanus*, 12 *annularis*, 2 *philippinensis*, 1 *jeyporiensis*, 1 *aconitus* and 1 *barbirostris* had been taken in one night. The total catch was 2 *A. minimus*. This is the only station from which there are comparable records at two seasons from which a measure of the reduction of the total population during the dry period can be gained. It was not possible to look at every luwin but all those seen, including some of the largest, were quite dry, except one in which water was confined to a few small pools.

A second night was spent near one of the Kale Valley villages on the banks of the main river flowing southwards. A complete night catch was not made but the impression was that *A. minimus* was as numerous as in the previous December. Males were found indoors and the total catch was 27 females and 12 males; this was the first occasion on which males of this species had been taken in a night catch. Other species were :—*A. annularis*, *A. maculatus*, *A. stephensi*, 1 female each; *A. vagus* 3 females, 1 male; *A. culicifacies* 6 females and 12 males. This was the first time that *A. culicifacies* had been encountered except in small numbers; it was breeding in the river which was now sluggish and in places much overgrown with pond weed and filamentous green algae.

In May 1945 a party was sent out to make further spraying experiments with DDT. Unfortunately it could not work in any region where observations had been carried out the year before, as both the Tamu area and most of the Kale Valley had come within the sphere of action of malaria control units, and eventually it settled in the Kabaw Valley some 5 miles south of the most southerly point reached in September the year before. Four villages were investigated. All were at the edge of the forest and conditions were strictly comparable with those of the forest stations of the previous year. The results of six nights' catching from 1–7 May before any spraying was done were *A. minimus* 701, *A. aconitus* 2, *A. culicifacies* 1, *A. maculatus* 1, *A. vagus* 1, and *A. stephensi* 1.

A. minimus completely dominates the picture at this time of year.

No larval catches were made but larval records for the early part of the year are available for 1944 when three Anti-malaria Units were operating round Tamu.

No. 20 Anti-malaria Unit, in January 1944, recorded 226 *A. minimus* and 198 *A. maculatus* from the Lokchao river and their records for collections in luwins are *A. barbirostris* 293, *A. aconitus* 86, *A. philippinensis* 17, *A. hyrcanus* 14, *A. annularis* 26, *A. stephensi* 9, and *A. jeyporiensis* 2.

The other two Units had, unfortunately, the reprehensible habit of expressing their figures as percentages without giving any indication of the total on which the percentage was based, so their results are of less value. They agree in general with those of No. 20 Anti-malaria Unit though they found *A. maculatus* to be more abundant than *A. minimus* in the river and neither found *A. aconitus* or *A. jeyporiensis* in the luwins. This, however, is not surprising as both species can easily be overlooked by an observer who does not know where to find them. Apparently no larval collections were recorded after February.

It is interesting to contrast the larval population of luwins in September and in January. In September *A. hyrcanus* was by far the most numerous species, its numbers being about double those of all the other species put together. In January it is relatively much less abundant and the common species is *A. barbirostris*. *A.*

aconitus is also relatively more important in January than in September. But the total number of specimens in a given area is much less in January and it probably decreases steadily from about November onwards as the breeding places become progressively fewer and smaller. The stream breeders, *A. minimus* and *A. maculatus*, are the only species for which extensive breeding facilities persist during the dry period of the year.

Table X shows the results of adult collections made by No. 20 Anti-malaria Unit in January, February and March.

TABLE X.

Adult collections in the Tamu area by 20 A.M.U.; day catches, 1944.

Month	*maculatus*	*minimus*	*barbirostris*	*annularis*	*philippinensis*	*stephensi*	*kochi*	*vagus*	*aconitus*
Jan. ...	246	128	104	16	7	—	—	—	2
Feb. ...	280	195	98	36	14	11	7	5	—
Mar. ...	476	245	125	84	45	38	26	—	—
Total ...	1,002	568	327	136	66	49	33	5	2

The village of Tamu at that time was intact. It is not possible to explain why numerous *A. maculatus* were indoors at Tamu but not in the Kale Valley in villages located close to a river in which larvae were abundant.

To summarise it is not possible, from the evidence which has been presented in the preceding pages, to tabulate exact data showing the seasonal abundance of the various species but it is possible to gain a reasonably accurate impression. During the rains many species are to be found and among the common ones are :—

As adults attacking man :—*minimus*, *philippinensis*, *leucosphyrus*, *jeyporiensis*, *annularis*, *hyrcanus*, *aconitus*.

As larvae :—*philippinensis*, *leucosphyrus*, *hyrcanus*, *barbirostris* and *vagus*.

A. minimus at this time is only locally abundant ; *A. leucosphyrus* is widespread but confined to forest areas.

When the rains cease the stagnant water breeding places begin to dry up rather rapidly. Small places soon disappear and the numbers of those species, such as *leucosphyrus* and *vagus*, which breed in them, fall immediately, though in certain localities, where there are suitable alternative breeding places in stream beds, a population may persist for longer. Larger breeding places do not disappear so quickly but only a few hold water until the next rainy season, and in general there must be a steady reduction in the numbers of species which breed in them throughout the dry period. At first *A. hyrcanus* and *A. philippinensis* are the two most abundant species and they both probably reach a maximum within a month after the cessation of the rains. Thereafter their numbers are reduced and certain changes in the composition of the larval population take place ; *A. barbirostris* becomes the commonest species and *A. aconitus* is taken in larger numbers than at other seasons. It was unexpected to find that *A. jeyporiensis*, though present in the late months of the year, was not found abundantly by the Anti-malaria Units in January. It was scarce in both the adult and larval collections. Evidence from the former is indefinite because all the catching was done by day but there is no reason to doubt the validity of the larval collection, as large numbers of *A. aconitus* were taken and the two species occur in similar places. In the Arakan *A. jeyporiensis* is abundant in the early months of the year.

As soon as the streams and rivers settle down, after the end of the rains, breeding facilities for *A. minimus* and *A. maculatus* become very extensive and both species increase correspondingly. Some of the smaller streams dry up and the weather becomes colder so the total population must decrease as the dry season progresses but in general both species abound between October and June.

The Vectors.

There was insufficient time for an examination of stomachs for oocysts, but the results of dissections for the presence of sporozoites are shown in Table XI.

TABLE XI.

Dissection results.

Place and occasion	Month (approx.)		*minimus*	*philippinensis*	*leucosphyrus*	*jeyporiensis*	*annularis*	*hyrcanus*	*aconitus*	*barbirostris*	*kochi*	*tessellatus*	*culicifacies*	*maculatus*
Tamu: Survey and DDT spray. Sprayed stations before spraying (Tables III & IV)	Sept.	Number dissected ...	332	160	134	55	106	12	24	8	4	3	4	2
		No. with sporozoites	1	—	2	—	—	—	—	—	—	—	—	—
		Sporozoite rate per cent.	0·30	—	1·49	—	—	—	—	—	—	—	—	—
Tamu. DDT spray. Sprayed stations after spraying	Oct.	Number dissected ...	95	124	9	41	26	232	4	18	—	—	—	4
		No. with sporozoites	1	—	—	—	—	—	—	—	—	—	—	—
		Sporozoite rate per cent.	1·05	—	—	—	—	—	—	—	—	—	—	—
Tamu. DDT spray. Control stations (Table VII)	Oct.	Number dissected ...	64	221	25	68	87	296	8	11	8	—	—	—
		No. with sporozoites	—	—	—	—	—	—	—	—	—	—	—	—
Kale Valley.	Dec.	Number dissected ...	519	—	—	—	2	—	5	—	1	1	—	—
		No. with sporozoites	19	—	—	—	—	—	—	—	—	—	—	—
		Sporozoite rate per cent.	3·66	—	—	—	—	—	—	—	—	—	—	—
Htinzin area.	May	Number dissected ...	514	—	—	—	—	—	1	—	—	—	1	—
		No. with sporozoites	10	—	—	—	—	—	—	—	—	—	—	—
		Sporozoite rate per cent.	1·94	—	—	—	—	—	—	—	—	—	—	—
Totals		Total dissected ...	1,524	505	168	164	221	540	42	37	13	4	5	6
		Total with sporozoites	31	—	2	—	—	—	—	—	—	—	—	—
		Sporozoite rate per cent.	2·03	—	1·19	—	—	—	—	—	—	—	—	—

Clearly, in the period between the rains, *A. minimus* is the important vector and is the only species transmitting malaria. But during the rains this species has a low sporozoite rate (0·3 per cent.) and furthermore it is not universally abundant. Another vector, perhaps playing the major part in transmission, is likely and there seems good reason to postulate that *A. leucosphyrus* with a sporozoite rate of 1·49 per cent. and a rather wide distribution in the forest is this vector.

There are, however, other factors, besides the sporozoite rate, that call for examination. The specimens dissected in December and May had been feeding on a static autochthonous population, but in September and October there was no local population available and the mosquitos examined in those months had been feeding on soldiers who did not belong to the valley. Most of the troops were Africans and it was noticed by the author, and confirmed by a number of officers, that the African soldier was very punctilious about taking the anti-malaria drug mepacrine. Whether on account of this, or of a natural immunity, is immaterial to the present discussion, the important fact is that malaria casualties in 11th Division were low and therefore the chances of the mosquitos becoming infected were reduced. It was significant that the infections found in *A. minimus* in September and October and one of the two infections found in *A. leucosphyrus* in September all came from the same camp, occupied by an Indian Unit whose malaria discipline was conspicuously deplorable and whose malaria rate was high.

It was reckoned that the collectors were always at least a month behind the main body of the Japanese forces, which at that time were in retreat, so that any infections acquired from this source would have largely disappeared. Furthermore, malaria casualties were removed to hospital outside the Kabaw Valley. The chances, therefore, of the mosquitos becoming infected were reduced, so although large numbers of some species were dissected and no infections were found, the possibility that these species might play a part in malaria transmission cannot be entirely ruled out.

When both adult and larval collections were made in the same area (Tables II—V) the numbers of *A. minimus* and *A. leucosphyrus*, the two species conclusively incriminated as vectors, taken by night near man were disproportionately large compared with the number of larvae and this suggests that these species were definitely attracted to man and converged on the camps from all breeding places for some distance around. The same holds for *A. jeyporiensis*. The disproportion is less great in the case of *A. philippinensis*, *A. annularis*, and *A. hyrcanus*. In an area where probably but a fraction of the total breeding places were located, no precise significance can be attached to the actual ratio of adults and larvae caught but it probably gives a rough indication of the extent to which each species was attracted to congregations of man. All the species mentioned were found in camps in large numbers so not one of them can be absolved from suspicion without the sifting of further evidence. An examination of the number of specimens of each species found to contain fresh blood was carried out and the results are presented in Table XII. This includes only the observations made in September and October since it was only in these two months that most of the species under discussion were taken in large numbers. The single collection made in an inhabited village (Hesin, see Table III) has been omitted to make the data discussed more uniform. Although, as noted above, cattle were sometimes found wandering in the forest the chances that a mosquito had fed on a cow in the forest and then sought shelter in a tent within a camp can be ignored ; fed specimens contained human blood.

Observations made during the course of the DDT air spray experiments are presented separately as it has been stated that contamination with DDT affects a mosquito's desire to feed.

The civilians were a group of Burmese who were living in one of the few houses in Tamu still habitable and helping with the reconstruction of the village. They had been given army mosquito nets.

TABLE XII.

Mosquitos containing fresh blood ; specimens caught at night near man.

Place and occasion	Month		*minimus*	*philippinensis*	*leucosphyrus*	*jeyporiensis*	*annularis*	*hyrcanus*	*aconitus*	*barbirostris*	*kochi*
Tamu Survey	Sept.	Number examined ...	363	142	128	61	126	66	25	14	5
		Number with fresh blood	33	13	29	5	2	1	—	1	—
		Per cent. with fresh blood	9	9	23	8	1·5	1·5	—	—	—
Tamu DDT spray. Military camps before spraying	Sept./Oct.	Number examined ...	33	24	45	29	17	27	—	—	—
		Number with fresh blood	9	—	8	3	1	1	—	—	—
		Per cent. with fresh blood	27	—	18	10	6	4	—	—	—
Ditto after spraying	Oct.	Number examined ...	58	60	21	14	16	250	—	—	—
		Number with fresh blood	18	7	3	1	1	8	—	—	—
		Per cent. with fresh blood	31	12	14	7	6	3	—	—	—
Tamu DDT spray. Control stations	Oct.	Number examined ...	44	196	18	48	81	382	—	—	—
		Number with fresh blood	12	28	4	2	5	13	—	—	—
		Per cent. with fresh blood	27	14	22	4	6	3	—	—	—
TOTAL (all observations in military camps)		Total examined	498	422	212	152	240	725	—	—	—
		Total with fresh blood ...	72	48	44	11	9	23	—	—	—
		Per cent. with fresh blood	15	11	20	7	4	3	—	—	—
Tamu DDT spray. Civilians before spraying	Oct.	Number examined ...	65	62	37	—	14	10	22	—	—
		Number with fresh blood	34	16	18	—	8	5	15	—	—
		Per cent. with fresh blood	52	26	49	—	57	50	68	—	—
Ditto after spraying		Number examined ...	64	99	6	38	15	69	4	—	—
		Number with fresh blood	32	32	2	13	7	14	3	—	—
		Per cent. with fresh blood	50	32	33	34	47	20	—	—	—
TOTAL civilians		Total examined	129	161	43	38	29	79	26	—	—
		Total with fresh blood ...	66	48	20	13	15	19	18	—	—
		Per cent. with fresh blood	51	30	47	34	52	24	69	—	—

Several interesting points come out of these figures. In September, during the rains, the proportion of *A. leucosphyrus* that succeeded in obtaining a meal, in spite of the use of mosquito nets, was more than twice that of any other species. *A.*

leucosphyrus was the most important vector at that time and in October the percentage was still fairly high. *A. minimus* had a low percentage in September but in October the percentage had jumped up and was usually higher than that of any other species. In September the sporozoite rate was low but in later months elsewhere it was high and October may well have been the month when an increase took place. It is not proposed to erect a theory on one set of figures, but the observation may be noted as one which might repay further study. Various factors must be concerned, but presumably the number of specimens that succeed in obtaining a meal when thwarted by a net depends on the extent to which they are stimulated to efforts to find a way into the net or locate a piece of flesh pressed against or protruding from underneath it. This may vary with physiological condition at different times of year and have a bearing on malaria transmission.

The total percentage of specimens that had been successful in their search for a meal of blood was, excluding for the moment those which had been feeding on the civilians :—*A. philippinensis* 11 per cent., *A. jeyporiensis* 7 per cent., *A. annularis* 4 per cent. and *A. hyrcanus* 3 per cent. These figures are lower than those for *A. minimus* and *A. leucosphyrus*, the two species definitely incriminated as vectors, but nevertheless these four species do bite man ; when the results obtained at the station inhabited by civilians, who, though in possession of mosquito nets, were not subject to military discipline and the anti-mosquito precautions which it enforced, are considered, it is found that the numbers biting man are high. Under war conditions, therefore, any of the four and, in addition, *A. aconitus* might play some part in malaria transmission. In the normal conditions of civilian life this conclusion does not necessarily apply as some or all of the species are, in all likelihood, primarily feeders on cattle and evidence relating to this question will now be discussed. Unfortunately there are no data on which an opinion on *jeyporiensis*, *annularis*, and *hyrcanus* can be based, but there is some further information about the feeding habits of *philippinensis*. Staff Sgt. Shepherd and Sgt. Mortimer captured a pony which had been left behind by the Japanese and on this one evening, in the course of an hour, they caught forty specimens of *A. philippinensis*, although, in the camp nearby, the total nightly catch at that period never reached double figures. On the other hand at New Hesin during the course of a night catch, which is described later on p. 258, it was found that *A. philippinensis* was attacking man, although cattle were present in the village ; therefore, in spite of 505 negative dissections, it is felt that the possibility that this species plays some part in malaria transmission in settled times cannot be wholly ruled out.

Incidentally the figures quoted in Table XII give an interesting index of the protection afforded by a mosquito net. What the civilians did with theirs was, unfortunately, not put on record but they did not get much protection from them, nor were personnel under military discipline obtaining as much protection as was commonly supposed, for when collecting was done in military camps it was, as Table XII shows, usual to find that some 20 per cent. of the vector species had discovered a body with some limb projecting from beneath the net or some part pressed against the net, and had obtained a meal. Nor can this 20 per cent. be taken as a minimum figure, for it is to be presumed that of the 80 per cent. which was captured before it had fed a proportion would have found a feeding place if left at large.

A. maculatus though abundant does not apparently attack man in this region and plays no part in malaria transmission.

Discussion on the status of A. leucosphyrus *as a vector.*

The conclusion is that during the rains in the Kabaw Valley *A. leucosphyrus* is a vector of major importance and, as it has been found infected at three other places near the Indo-Burmese border, there is good reason to believe that this conclusion applies to a much wider area. The other records of infection are :—

(1) Clark and Chowdhury (1941) recorded a sporozoite rate of 2·4 per cent. in 859 dissections at Digboi, in the Brahma Putra Valley near Ledo. Dr. R. H. P. Clark, in reply to a letter from Lt.-Col. G. I. Watson, kindly gave some additional information and stated that, since the publication of the paper, *A. leucosphyrus* had been found infected in all the months from June to October inclusive.

(2) Covell (1944) refers to a record of infection on the Ledo road. Unfortunately we were not in touch with the American malariologists and have no details of their findings. Dr. Clark, however, in a letter to me remarks that this species was repeatedly found infected by the Americans. It is to be hoped that an account of their work will soon appear in print.

(3) Lt.-Col. C. G. Terrell, O.B.E., E.D., found one specimen with sporozoites out of 92 dissected during the course of investigations carried out while he was Chief Medical Officer to the Labour Force building the Aijal road, which ran from the plain of Bengal into the Lushai hills between Chittagong and Imphal (see fig. 1). These findings were published in a typed report circulated to malariologists and Lt.-Col. Terrell has kindly supplied additional information in subsequent correspondence.

Previously *A. leucosphyrus* had not been regarded as a vector of great importance, but recently McArthur (1946) has brought forward evidence that it is a major vector in Borneo, and Toumanoff (1936) in Indo-China writes (p. 414) :—" Il est fort probable ainsi que cette espèce, quoique rare, constitue, grâce à l'intimité de ses rapports avec l'homme, un vecteur du paludisme assez important."

The status of this species in the western parts of its range calls for further examination and the following points seem relevant :—

(1) The species appears to be confined to forest as it is in Borneo (McArthur, 1946). Clark and Chowdhury noticed that, though malaria among the oil field coolies had been greatly reduced by the usual measures directed against *A. minimus*, the disease persisted among woodcutters and other persons dwelling in the forest. They followed up this clue and incriminated *A. leucosphyrus*. Its absence from clearings in the Kabaw Valley has already been described and it is noteworthy that Watson, working south of Myitkina at a time when the species was being incriminated in the Kabaw Valley, failed to secure it. Since it was found further north along the Ledo road by the American workers, he was undoubtedly within the range of this species. Careful analyses of his numerous night catching stations revealed that not one of them was properly in the forest and this appears to be the explanation of why he did not record *A. leucosphyrus*. The conclusion is that *A. leucosphyrus* was probably of great importance as a vector during military operations, when large bodies of troops were occupying forest areas, but that it is of limited importance as a carrier of civilian malaria since the proportion of the population which inhabits the forest is not large.

(2) Evidence presented below (p. 257), though not conclusive, suggests strongly that *A. leucosphyrus* does not rest indoors.

Clark, in a letter, writes " In Digboi most of our captures (all females) were made in defective mosquito nets." Possibly a gorged female makes less strenuous efforts to find a hole in a mosquito net than she did when she was outside and hungry. If this hypothesis be correct a defective net would act to some extent as a trap, and Clark's findings bear out that *A. leucosphyrus* is not a house haunting species. Possibly, therefore, this species, of which the breeding places are not always easy to find, has been overlooked where reliance has been placed on day catches and it may be of wider importance in civilian life than has hitherto been recognised. McArthur

(1946) is of the opinion that it has escaped incrimination in Borneo for so long because it does not stay in houses after feeding.

(3) On the other hand, evidence that *A. leucosphyrus* increases in abundance as a result of military activities, though scanty, is definitely worthy of notice. Ramsay, Chandra and Lamprell (1936) conclude from observations on feeding habits that *A. leucosphyrus*, where prevalent, would be a dangerous vector. Dr. C. G. Ramsay, C.I.E., O.B.E., has kindly communicated further information in a letter in which he writes " this species is relatively rare on tea estates throughout Assam, except on a few estates in the Cachar district where, owing to the partial opening up of heavy jungle for gun sites, etc., during the recent war the species greatly increased."

Terrell's observations point to a similar conclusion. The Aijal road runs from Loharbund to Sairong via Aijal, a distance of approximately 108 miles. The first stretch of road, about 27 miles long, is at an altitude of some 800 feet and is highly malarious. The road then follows the crest of a ridge and rises steadily to an altitude of 3,500 feet 56 miles from Loharbund. It crosses a valley, of which the lowest point is 1,200 feet above sea level, and then follows the crest of a second ridge, climbing steeply to 4,500 feet, subsequently dropping gently to Aijal, which is 93 miles from Loharbund and 3,450 feet above sea level.

Terrell's findings may be summarised as follows :—

(1) Malaria is intense up to 2,000 feet. It is particularly associated with cultivation and *A. minimus* is probably the important vector.

(2) Above 2,000 feet malaria decreases with increasing altitude. The spleen rates are low and there is evidence that such malaria as is contracted is mainly acquired when the people go down to the cultivated valleys ; thus enlarged spleens are found chiefly among older children who have reached an age when they take part in cultivating.

(3) Malaria among troops and labourers was acquired to a large extent at lower altitudes. All the men had to walk to the road head and, in consequence, spent at least two nights in the lower and highly malarious stretches of the road. But malaria was also acquired at higher altitudes under conditions which suggested that *A. minimus* was not the most important vector.

(4) Infections found were :—*A. leucosphyrus* 1 sporozoite infection in 92 specimens ; *A. minimus* 1 oöcyst infection in 58 specimens. Both species were found at all altitudes but *A. leucosphyrus* was the commoner at higher altitudes.

(5) The studies on the local population already mentioned suggest that under normal conditions there is little malaria contracted except near the cultivated areas and this leads to the conclusion that *A. leucosphyrus* is of no great importance.

(6) It has to be inferred as a further step that the activities of road building caused *A. leucosphyrus* to become more important and that it carried malaria to personnel camped on the ridge in situations where villages in peacetime were relatively free. In the Kabaw Valley slit trenches and other places resulting from military occupation frequently harboured a good population of *A. leucosphyrus*, but as has been explained already, it was not possible in the forest to cover even a small area in such a way as to make possible an assessment of the relative importance of such artificial to natural breeding places.

Notes on Resting Places.

In the Tamu area in September it became apparent quite early on that catches made by day, whether in tents or in the one house still intact in the village, were likely to be fruitless. Night catching was started, therefore, and good catches were obtained in places where there were no mosquitos by day. When the party started work in the Kale Valley in December catches by night were made as a matter of course until it was noticed that, in contrast to what had been found at Tamu, a fair proportion of specimens had old blood in the stomach and partly developed or ripe eggs. This suggested that the mosquitos were resting in houses after feeding and that day catching would be profitable; this proved a correct forecast and all the specimens required for dissection could be collected by day. On the other hand, in the Kabaw Valley it was necessary to revert exclusively to night catches in May. Evidently there was a difference in resting habits with place or time which was likely to be interesting and of importance in malaria studies. Long searches in houses both by day as well as by night did not seem to be the most satisfactory way of investigating this problem, and it became evident that the best method of obtaining comparative figures for the extent to which mosquitos were resting indoors by day was to catch by night and find the percentage of specimens which had fed on an earlier night. Results from different places are shown in Table XIII.

TABLE XIII.

Specimens with old blood caught indoors by night.

Place and occasion	Month		*minimus*	*philippinensis*	*leucosphyrus*	*jeyporiensis*	*hyrcanus*	*annularis*	*aconitus*
Tamu Survey	Sept.	Number examined	366	177	128	63	66	136	25
		Number with old blood	31	0	1	2	0	0	5
		Per cent. with old blood	8	—	1	3	—	—	20
Tamu DDT spray. Control stations	Oct.	Number examined	44	196	—	—	382	—	—
		Number with old blood	2	2	—	—	3	—	—
		Per cent. with old blood	5	1	—	—	1	—	—
Kale Valley	Nov./Dec.	Number examined	223	—	—	—	—	—	—
		Number with old blood	87	—	—	—	—	—	—
		Per cent. with old blood	39	—	—	—	—	—	—
Htinzin area	May	Number examined	518	—	—	—	—	—	—
		Number with old blood	16	—	—	—	—	—	—
		Per cent. with old blood	3	—	—	—	—	—	—

In September and October nearly every mosquito caught had fed on the night of capture or, being without any blood and having small ovaries, had presumably entered to feed. Very few specimens were, therefore, spending the day in the places where they were found at night. Unfortunately most of the catching places were tents and so it is not possible to state how the various species behave when there are well-built inhabited native houses in which to rest. Nor are there figures for other months of the year for any species except *A. minimus*. It may be pointed out that in September and October a tendency to rest indoors was more marked in *A. minimus* than in any other common species.

The data for *A. minimus* are fuller and of some interest. In September and October and in May in the Kabaw Valley only a few specimens rest indoors by day; in December in the Kale Valley a much higher percentage is to be found in houses in the daytime. It is particularly unfortunate that it is not possible to separate the factors of time and place; it is thought likely that when forest comes up to the edge of the village, as in the Kabaw Valley, *A. minimus* leaves the houses before dawn and seeks a resting place in the forest, but where, as in the Kale Valley, open cultivated land surrounds the village then *A. minimus* seeks daytime shelter in the houses where it has fed. But it may be that *A. minimus* rests outdoors in the warmer parts of the year and tends to remain in houses during the colder weather. One definite assertion can be made and that is that the common belief that *A. minimus* is a house rester requires modification. One further lesson to be learnt from the data so far presented is that collections made by night are of the utmost importance and day catches may give a quite false picture of the mosquito population.

In one village both day and night collections were made in the same houses. By night it was found that though the population density differed from house to house it could not be correlated with any obvious feature of the house and it was probably related to distance from the breeding area. By day it was found that the houses which harboured mosquitos were those which were well built with dark inner rooms. The light and airy houses walled with bamboo matting had few mosquitos unless there were such resting places as old tins, earthenware jars, rolled up mats and accumulations of junk.

Rate of Ovarian Development.

It was found in the Kale Valley in December that the specimens caught both by day and by night fell clearly into 4 groups according to the stage of development of the ovary. As the separation into groups was clear cut it was deduced that each one represented 24 hours development and confirmation was obtained when a catch was dissected on two successive days and the proportions of each group on successive days compared.

The course of events inferred from these findings was as follows:—

On the first night the mosquitos fed and by the morning the ovaries had nearly completed stage 2; on the second and third nights the mosquitos rested and the ovaries developed to stages 3 and 4 respectively; on the fourth night most specimens also rested and the ovaries reached maturity; and on the fifth night the eggs were laid. The proportions of the four groups bore a rather constant numerical relationship to each other, each succeeding group being about half as numerous as the one preceding. Some mortality due to spiders and other causes is to be expected during the days when the mosquitos are indoors, but it is suggested that the reduction is largely due to the fact that the mosquitos move about each night and a certain proportion finally settle down in outdoor resting places and do not enter houses again until they have laid eggs and come back to feed.

Further Observations at Night.

Two nights spent collecting yielded observations which seem worthy of a brief description. The first was spent in the village of Hesin and yielded a total of *philippinensis* 31, *annularis* 8, *jeyporiensis* 2, *minimus* 2, *culicifacies* 4 and *maculatus* 1. The five specimens belonging to the last two species were found near an ox tied up underneath a house. The rest of the catch, which was predominantly *A. philippinensis*, was caught actually biting the human population. A large crowd gathered on the veranda of the headman's house and a few people slept there all night; it was here that all the specimens were caught. No specimens were found inside the headman's house nor in any of the other three houses visited at intervals throughout the night. The veranda was a wide platform with no roof and behind it was the large open front room of the house. Lt.-Col. G. I. Watson, working with repellents in the Arakan, also noted that *A. philippinensis* attacked people sitting out of doors.

The second night of special interest was in the village of Tamu. A single house, well made of wooden planks, was the only undamaged house, most of the rest having been burnt to the ground, and this was occupied for the night by a party of three collectors and four African guards. Two guards kept watch outside the house and the other two slept inside. As far as was known there were no other humans in the vicinity though Japanese stragglers were occasionally reported. Exposed parts of the body were protected by repellents, the Europeans using dimethyl phthalate and the Africans citronella ointment.

The night's catch was *A. minimus* 69, *A. philippinensis* 46, *A. leucosphyrus* 18, *A. jeyporiensis* 14, *A. aconitus* 14, *A. annularis* 10, *A. barbirostris* 2, and *A. vagus* 1. About half of the specimens were found sitting quietly on the outside walls of the house; they were found all round the house and were not concentrated near a sentry. No violent efforts to bite through the clothing, which was not impregnated with any repellent, were noted, though 5 specimens which had managed to obtain a meal were caught. All the mosquitos disappeared with the first light of dawn. The behaviour of *A. philippinensis* under these circumstances was quite unlike that at Hesin where it was not found resting anywhere either before or after feeding. The impression gained from all the nights spent collecting was that all species tended to rest for an appreciable period near the source of food before making any attempt to feed but unfortunately it was not possible to estimate this period with any degree of accuracy.

The investigation immediately preceding the author's arrival in the Kabaw Valley had been in a coastal district where *A. sundaicus* was the chief Anopheline and the contrast between the behaviour of that species and the behaviour of all the mosquitos at Tamu was striking. *A. sundaicus* also rested for some time in the immediate neighbourhood of its prey before attacking but, when it attempted to feed and found itself prevented by repellents and protective clothing, some specimens made what can only be described as frantic efforts to find an uncovered piece of body and a few lurked about well after sunrise and attacked when the protective clothing had been removed.

Notes on Time of Activity.

All the times quoted in this section have been corrected to local time.

A. minimus.—During September, at which time of year it is sufficiently accurate, for present purposes, to say that nights and days were of equal length, four nights' observations were made at Moreh and, although the catchers were not the same each night, the results are comparable as collecting was carried out fairly continuously.

At Moreh Bridge Camp, Tamu, on 4th–5th September, 6 were caught before 23.00 hours, 10 between 23.00–02.00 hours and 19 after 02.00 hours. On the next three nights, 18, 8 and 15, respectively, were caught before 01.00 hours and 30, 70 and 3, respectively, after 01.00 hours.

Immediately before Tamu Village was sprayed with DDT, catching was begun at 22.00, 24.00, 02.00, 04.00 and 06.00 hours and the numbers taken on 30th September–1st October were 2, 0, 6, 8 and 18 respectively and the following night 2, 6, 8, 1 and 0 respectively.

In several smaller catches more specimens were taken before 01.00 hours than later.

The conclusion is that the time of activity is rather variable; it is usually during the early hours of the morning but sometimes it is in the first half of the night and the proportions of the catches at different times show a wide range of variation.

In the Kale Valley in December only one night's complete catch was made. The greatest number was taken between 23.00 and 02.00 hours and on subsequent nights catches made between these times were always good.

In the Kabaw Valley in May the population was almost pure *A. minimus* and there were very few specimens with old blood. Mosquitos began to come in at about 22.30 hours which would be roughly four hours after sunset and the catches are shown in Table XIV.

A number of specimens was taken before 01.00 hours but the heaviest attack was always in the latter half of the night. In this respect the behaviour was more constant than in September but there was still considerable variation in the time of the greatest attack. In May climatic conditions were fairly stable ; in September the rains were still in progress but no correlation between the activity of *A. minimus* and any climatic factor was noticed. The party had no meteorological instruments at its disposal. Mosquitos were observed to be active during heavy rain.

The percentage of the catch which had fed at different times was extremely variable.

TABLE XIV.

Numbers of *A. minimus* caught at different times of night. Villages Htinzin area.

Place	Date					Remarks
Old Htinzin	May 1/2	Times ...	21.30–24.00	24.30–01.30	01.45–05.30	Noticeably scarcer after 04.30 hrs.
		Catching period	2½ hrs.	1 hr.	3¾ hrs.	
		Number caught	28	15	60	
Old Malu	May 2/3	Times ...	22.00–02.00	02.00–04.00	04.00–06.00	
		Catching period	4 hrs.	2 hrs.	2 hrs.	
		Number caught	13	24	25	
New Malu	May 2/3	Times ...	22.00–02.00	02.00–04.00	04.30–06.30	
		Catching period	4 hrs.	2 hrs.	2 hrs.	
		Number caught	49	79	28	
Old Htinzin	May 3/4	Times ...	22.00–01.00	02.00–04.00	04.00–06.00	
		Catching period	3 hrs.	2 hrs.	2 hrs.	
		Number caught	24	12	44	
New Htinzin	May 3/4	Times ...	22.00–01.00	02.00–04.00	04.00–06.00	
		Catching period	3 hrs.	2 hrs.	2 hrs.	
		Number caught	38	153	11	
Old Malu	May 6/7	Times ...	23.00–00.15	00.30–02.00	02.30–04.00	
		Catching period	1¼ hrs.	1½ hrs.	1½ hrs.	
		Number caught	13	33	53	

A. leucosphyrus.—Fewer observations were made on this species but it is so distinct that it was possible to record more accurate data. It appeared to behave with greater regularity than any other species. It was usually first observed at about 22.00 hours and was fairly numerous an hour or so later; totals before and after 01.00 hours were usually about equal, then it disappeared rather suddenly and it was rare in catches after 03.00 hours.

A. philippinensis.—Reference has already been made to the night spent at Hesin (see p. 258), where mosquitos were attacking people sitting on the veranda of a Burmese house. Work, unfortunately, did not start until 22.30 hours on 29th–30th August, and catching from then on was continuous until 06.00. The numbers caught in each successive half-hour were 15, 7, 7, 1, 0, 10, 6, 3, 3, 1, 2, 2, 1, 2, and 0, respectively.

This total of 60 is the number of mosquitos which the observers thought they caught; the actual number of specimens in the cage next day was 48. *A. philippinensis* predominated to such an extent that its behaviour may be deduced from the total figures. There was a marked peak of biting intensity between 22.30 and 23.00 and thereafter a marked falling off; a second peak occurred between 01.00 and 01.30 and biting continued at a reduced rate from then until dawn.

Further observations were made in September and October. During the night catch at Tamu, 31st August/1st September, it was noted that the first mosquito was seen at 20.50 hours and the first capture was made at 21.25 hours, and that, to begin with, the majority of the specimens had white tips to the hind legs. *A. philippinensis* and *A. annularis* were the only species having this character, which were found in the catch next day and, as their respective totals were 46 and 10, it was evidently *A. philippinensis* that predominated at the beginning of the night.

At Witok on the night of 18th–19th September, 8 were caught between 21.40–22.30 hours, 5 between midnight–00.45, 2 between 02.05–02.25, and 0 between 04.35–05.00.

At Tamu Village on the last night before spraying was started (30th September–1st October), catching began at 22.00, midnight, 02.00, 04.00 and 06.00 when the numbers taken were 1, 3, 8, 1 and 6, respectively.

After the spraying, when the mosquito population was beginning to recover, *A. philippinensis* was one of the commonest species though the numbers were not large compared with catches in areas that had not been treated. In catches made on 9 nights at the same 5 times at Tamu Village in October, the numbers taken were largest at the first collection on 2 of the nights, at the second on 5, at the third on 1 and at the fourth on 1.

Numbers of *A. philippinensis* caught on a pony at Tamu Village on 8th–9th October were 34 between 19.45–20.00 hours, 6 between 20.30–20.45, 0 between 20.45–21.00 and 1 between 00.45–01.00.

Sunset on this date was about 17.40 hours.

In the Kabaw Valley, therefore, in September and October *A. philippinensis* was active throughout the night but it appeared before *A. minimus* and *A. leucosphyrus*. The period of greatest activity was variable but was frequently before midnight. Other members of the unit working in Bengal at the same time found that *A. philippinensis* was taken in greatest numbers after midnight.

A. hyrcanus was the earliest species of all and was entering within two hours of sunset. Frequently its numbers were high when the first collection was made and low during the rest of the night but, like the other species, it was variable in its behaviour, and the variation could not be correlated with any obvious climatic factor. On one night when a particularly big catch was obtained and careful records were kept the greatest number was taken between 03.00 and 03.30.

The party was constantly alert for mosquito attacks by day for a favourite excuse among units called upon to explain heavy malaria casualties was that the men had been bitten in the forest during the day when no protective measures were in force. We found that no species had a habit of biting by day though very occasionally a single *A. barbirostris* might attempt to feed while the sun was up and once a specimen of *A. annularis* was caught in this act.

Attraction to Light.

During the spraying experiments in October, the officer's mess of the Section of No. 1 Malaria Field Laboratory consisted of a tarpaulin slung over a ridge pole. The ridge was some 10 feet from the ground and the tarpaulin sloped down to a height of about 4 feet at the outer edges.

An electric light was slung from the ridge pole. Each night, a great variety of insects gathered round the light soon after it was put on and among them were to be found a number of female *A. hyrcanus*. They were not near the edge in the darker places, where mosquitos were to be expected, but at the top in the most brightly illuminated area. Very few made any attempt to bite and out of a total of 241 specimens only 5 were found with fresh blood, 3 of them all on the same night. In another camp it was noticed that nearly all of a fairly large catch of *A. hyrcanus* were taken in one tent where a light remained on all night. There seemed therefore to be definite evidence that *A. hyrcanus* is attracted to light. Senior White, in a personal communication, writes " *A. hyrcanus* comes to light in numbers in Calcutta in October only. I have always associated this with emigration from drying rice-fields to permanent swamps. I have never known such to bite."

Systematic Notes.

According to the key of Puri (1941), the branches of the posterior clypeal hair of the larva number 2–5 in *A. pallidus* and 7–10 in *A. philippinensis* and this is one of the main distinctions between the two species. The same author (1931), describing the larvae more fully, gives the number of branches in *A. philippinensis* as 5–11. Many larvae taken in the Kabaw Valley had 5–6 branches to the posterior clypeal hair, and were named *pallidus* as the filaments of the palmate hairs were nearer half the length of the blade than quarter and the specimens therefore tallied more closely with *A. pallidus* on the second of the two characters given for separating these species. On emergence all proved to be *philippinensis*. The Anti-malaria Units recorded mostly *A. pallidus* in their larval collections, but only *A. philippinensis* in their adult collections. There is therefore, in this region, good reason to doubt the validity of all identifications of *A. pallidus* based on larval material and in the preceding pages all larval records of *A. pallidus* have been treated as *A. philippinensis*.

Considerable difficulty was experienced with species in what is commonly referred to as the *minimus-funestus* complex, although *A. funestus* is not an Indian species. A limited number of specimens, particularly from the later catches, was examined in detail so that a record could be made of certain features having systematic importance. The total number of mosquitos examined was small and the work was carried out under difficult conditions.

The differences between the four species in the complex are set forth in Table XV. The data are taken from Christophers (1933) and include in his own words the reservations which this author makes. It may be mentioned, incidentally, that these reservations are omitted from the key of Puri (1938). It will be seen that the first two differences depend on presence or absence of a spot and in order to simplify the subsequent discussion it is convenient to denote presence by a capital letter and absence by the same letter in lower case type, e.g. white spot at base of costa present =A, and white spot at base of costa absent=a. The letters are applied serially to the five parts of the insect which show differences in the different species. The third character (c) depends on a simple numerical difference and the same system is applicable. In the fifth instance there are three possibilities so Clarendon type may be introduced to indicate the third possibility. . . . .

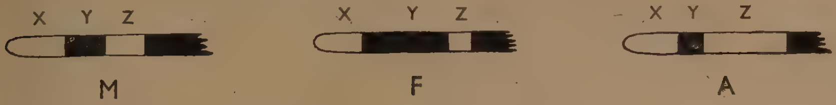

Fig. 2.—Schematic representation of the terminal portions of the palps of : M, *A. minimus* (YZ), F, *A. fluviatilis* (Y4Z) and A, *A. aconitus* (Z4Y).

The markings on the palps, which have been put in the fourth column of the table, are a little less straightforward but they too can be made to conform to the scheme without making it unduly complex. The differences lie in the relative proportions of the bands and a great deal of cumbersome phrasing can be avoided if these also be referred to by letters of the alphabet and the proportions indicated by a simple mathematical expression. Since the first letters of the alphabet have already been used, confusion will be avoided if the end of the alphabet be employed for the present purpose. The terminal band of the palp, which is white in all the species under discussion, may be referred to as X, the one next it, a black band, as Y and the one next again, i.e. the middle white band, as Z (fig. 2). Then instead of saying as Christophers does of *A. minimus* :—" the female palpi have two broad white bands apically, the subapical one usually as broad, or nearly as broad, as the apical and the dark area between the bands usually about the same extent as either of the pale bands, rarely exceeding twice the extent of the subapical pale band," and of *A. fluviatilis* :—" Palpi . . . black band between apical and subapical pale band usually four to five times length of subapical pale band," it is possible to express the information as, *A. minimus* YZ-Y2Z (fig. 2M) and *A. fluviatilis* Y4Z-Y5Z (fig. 2F). Palpal markings of three types are described by Christophers and these are denoted by the letter D of three different kinds. The Burma specimens, however, show a wider range than that described by Christophers and it is necessary to extend the limits of each category somewhat. The following divisions have been made :—

D. YZ-Y3Z.
d. Z2Y-Z5Y.
d. Y4Z-Y5Z.

TABLE XV.

Distinguishing features of species of the *minimus-funestus* group ; from Christophers.

Species	White spot at base of costa	Fringe spot opposite vein 6	Markings on vein 6	Palpal bands	Markings on proboscis
minimus ...	" very constantly " present (A)	" rarely " present (b)	" Usually " two black bands, the outer half being continuously dark (C)	YZ–Y2Z (D)	Dark (E) or with a ventral flavescent tache (e)
aconitus ...	" may or may not be " present (A) or (a)	presence " fairly constant " (B)	" usually " three black bands (c)	Y very small, may be absent **d**	whole of distal half of proboscis flavescent (**e**)

Species	White spot at base of costa	Fringe spot opposite vein 6	Markings on vein 6	Palpal bands	Markings on proboscis
fluviatilis ...	absent (a)	absent (b)	as *minimus* (C)	Y4Z–Y5Z (d)	Dark (E)
varuna ...	"invariably" absent (a)	absent (b)	as *minimus* (C)	YZ–Y2Z (D)	usually shows faint or sometimes marked flavescence over the outer half, above and below (**e**)

Thus typical specimens are :—

minimus	A	b	C	D	E	or e
aconitus	A or a	B	c	**d**	**e**	
fluviatilis	a	b	C	d	E	
varuna	a	b	C	D	E	or **e**

There is hardly one of these characters which, according to Christophers, is quite constant and does not occasionally conform in type to that of another species. I learn, however, from Major R. Senior White, with whom I had many helpful discussions, that experienced workers in India are apparently only rarely confronted with a specimen to which they cannot readily and with confidence put a name.

Toumanoff (1936), working in Indo-China, records in *A. minimus* a range of variation much greater than that recorded by the Indian workers and he has made an extensive analysis of a collection of 400 specimens from Tonkin of which the following is a summary :—

(1) Only 61 per cent. of specimens have a pale interruption at the base of the costa.
(2) 5 per cent. of specimens have a fringe spot opposite vein 6.
(3) The proboscis is dark in most specimens ; no figures are given.
(4) No variation in the palpal markings is mentioned.

In addition to the basal costal spot and the fringe spot opposite vein 6, many other characters of the wing vary and the combinations of these variations are such that no less than 24 types can be recognised.

Similar variation is found in *A. aconitus*, and Toumanoff concludes that the only constant difference between the two species is the flavescence of the proboscis.

Neither *A. fluviatilis* nor *A. varuna* is mentioned as occurring in Indo-China.

Turning now to the specimens captured in Burma, one series from the Kabaw Valley was as follows :—

A	b	C	D	E	8	(typical *minimus*)
A	b	C	D	e	18	(,, ,,)
A	b	C	**d**	e	8	(,, ,, palps of *aconitus* type)
A	b	C	**d**	E	3	(,, ,, ,, ,, ,, ,,)
A	B	C	D	e	4	(,, ,, except fringe spot present)
A	B	C	D	E	2	(,, ,, ,, ,, ,, ,,)
A	B	C	**d**	e	2	—
a	b	C	D	e	1	(*fluviatilis* wing, *minimus* palp)
a	b	C	D	E	1	(,, ,, ,, ,,)
A	b	C	d	e	2	(*minimus* wing, *fluviatilis* palp)
A	B	c	**d**	e	1	(typical *aconitus* except for proboscis)
A	b	c	**d**	e	1	—

No specimen shows all the characters of a typical *A. fluviatilis* or *A. varuna*, though four out of 51 show some of the characters of these species. All were accordingly named *A. minimus*. It would appear that variation in this species is much greater than is commonly found in India, in which respect the present material resembles that examined by Toumanoff. It is interesting to note, however, that whereas this author found the palpal characters to be constant and that most of the variation was on the wings, in the present instance the reverse obtains. The variation in the relative widths of the palpal bands could only be judged by the naked eye and recorded to the nearest whole number, but in spite of this rough and ready method it is felt that the results obtained were of sufficient significance to justify presentation. The results were :—

Y4Z	Y3Z	Y2Z	YZ	Z2Y	Z3Y	Z4Y	Y very small	Y absent
2	2	8	25	2	5	3	3	1

The flavescence of the proboscis was extremely well marked in some specimens and there was every gradation between this character and a uniformly dark proboscis. I have not, unfortunately, been able to see specimens of the Philippine *A. minimus* var. *flavirostris* but from descriptions it would seem that some Burma specimens must resemble it very closely.

Only a few specimens of *A. aconitus* from the Kabaw Valley were examined but a note may be inserted here on the comparison of *A. minimus* and *A. aconitus* from various parts of Burma. The presence or absence of a basal costal spot was very variable. More constant characters of *A. aconitus* were :—

(1) Fringe spot opposite vein 6.

(2) Three black marks on vein 6.

(3) Whole of the distal half of the proboscis flavescent.

(4) Outermost black band on the palp small.

The following departures from the typical form were found in 52 specimens.

(1) No fringe spot opposite vein 6—1.

(2) Two black marks on vein 6—7.

(3) Proboscis without characteristic flavescence—0.

Corresponding aberrations in 141 specimens of *A. minimus* were :—

(1) Fringe spot opposite vein 6—10.

(2) Three black marks on vein 6—2 (in 1 specimen associated with the presence of a fringe spot).

(3) Proboscis with flavescence as in *aconitus*—0.

The relative proportions in the two species were :—

	Y4Z	Y3Z	Y2Z	YZ	Z2Y	Z3Y	Z4Y	Z5Y	Y very small	Y absent
minimus ...	2	4	15	61	24	18	7	2	4	4
aconitus ...	0	0	0	0	1	4	10	3	16	12

Among the specimens from Burma, therefore, there is only one distinction between the two species and that is the flavescence on the proboscis ; this conclusion is the same as that reached by Toumanoff in Indo-China. The other characters, however, only break down in a relatively small percentage of cases.

Acknowledgements.

During most of the time I was attached to 11th (East African) Division and came in contact with many different Units. I received many kindnesses and much assistance and I am glad of the present opportunity to put this on record. My thanks are specially due to Major G. L. Broderick, R.A.M.C., commanding 21st (East African) Mobile Malaria Section.

Sgt. D. A. Mortimer, Pte. (later Sgt.) D. A. McLennan, Pte. J. B. Dorea and Pte. R. W. S. Rainsford of No. 1 Malaria Field Laboratory accompanied me on various occasions and I acknowledge gratefully the important contribution which their conscientious work made to the success of the investigation. I also wish to thank the various officers who were with me at different times and who have been named in the text.

Without the active support of Lt.-Col. J. Hay Arthur, M.B.E., I.M.S., on the staff first of 14th Army and later of Allied Land Forces South East Asia, much less could have been achieved.

I wish to pay a sincere tribute to Lt.-Col. G. G. Mer, O.B.E., R.A.M.C., Commanding Officer of No. 1 Malaria Field Laboratory, for under his direction I gained almost all the knowledge which enabled me to carry out the surveys described above.

Prof. P. A. Buxton, F.R.S., Capt. C. Diver, C.B.E., and Major R. Senior White, F.R.S.E., have kindly criticised the manuscript.

Summary.

(1) The Kabaw and Kale Valleys, separated by a low watershed and both draining through lateral gorges, have a combined length of some 250 miles and a width of between 4–10 miles. They lie at an altitude of approximately 500 feet above sea level.

(2) There is heavy rainfall during the months of June to September inclusive and little or no rain in the months November to April inclusive. The Kabaw Valley, the more northerly of the two, is forested and there are only small isolated clearings, but a big area in the centre of the Kale Valley has been cleared. Rice is the main crop grown.

(3) There appears to be no pre-war information about mosquitos and malaria. Information during the war was gathered by several different types of Unit engaged on different types of work but there is sufficient to give a fairly complete picture.

(4) The valleys are highly malarious throughout their length and breadth; only one village out of twenty-three examined had a spleen rate under 60 per cent.

(5) Transmission of malaria takes place throughout the year and is intense during the months May to December inclusive.

(6) 17 species of *Anopheles* are recorded. Common species during the rains are *A. minimus*, *A. leucosphyrus*, *A. philippinensis*, *A. jeyporiensis*, *A. annularis*, *A. hyrcanus* and *A. vagus*. *A. minimus* is only locally abundant at this time; *A. leucosphyrus* is widespread but confined to the forest.

(7) When the rain ceases, species such as *leucosphyrus* and *vagus*, which breed in pools and similar habitats, become reduced in numbers. Species such as *philippinensis*, *jeyporiensis*, *annularis*, *hyrcanus* and *barbirostris*, which breed in larger bodies of stagnant water such as rice fields, also become reduced in numbers during the dry season, though more slowly, because their breeding places disappear less rapidly. As the streams and rivers settle down, after the rains have stopped, conditions for *A. minimus* and *A. maculatus* become good and these are the only two common and widely distributed species in the dry season.

(8) During the dry season, sporozoite rates of *A. minimus* were 1/159=0·63 per cent. in October, 19/519=3·66 per cent. in December and 10/514=1·94 per cent.

in May and it is an important vector and the only vector. *A. maculatus* does not apparently attack man and plays no part in malaria transmission. In September the sporozoite rate of *A. minimus* was only 1/332=0·31 per cent. and the important vector of the period of the rains appeared to be *A. leucosphyrus* with a sporozoite rate of 2/134=1·49 per cent.

(9) The status of *A. leucosphyrus* as a vector in the Indo-Burmese border region is discussed. There is good evidence that, under wartime conditions with troops operating in the forest, it is an important vector during the rains. Its significance in peacetime is less easy to assess ; as only a few people live in the forest or at the forest's edge, its importance is probably not great. On the other hand it does not rest indoors, and, as in the past reliance has been placed on day catches, it may have been overlooked. There is, however, evidence that military operations in the forest produce conditions which lead to an increase in the numbers of this species.

(10) Adult collections were nearly all made at night in camps or villages. The species found in large numbers near man were *A. minimus*, *A. leucosphyrus*, *A. philippinensis*, *A. jeyporiensis*, *A. annularis*, and *A. hyrcanus*. *A. minimus* and *A. leucosphyrus*, the two definitely incriminated vectors, were the most successful at obtaining blood when the host, man, was protected by a mosquito net. The other four species, however, were sometimes filled with blood, undoubtedly of human origin and all may play some part in malaria transmission under war conditions. The chances of a specimen becoming infected were reduced under the conditions existing when the dissections were made and so moderately long series of negative dissections (Table XI) cannot be taken as conclusive evidence that any one species played no part in malaria transmission. In peacetime these four species may feed mainly on cattle, though a description is given of a night catch in a village where *A. philippinensis* was caught attacking man although cattle were available.

(11) In September very few of any of the eight common species were resting by day in the place where they fed at night and it is presumed that they spent the day in the forest. Unfortunately very few inhabited houses were visited and almost the only available catching places were Army tents and shelters. The results suggest, however, that all the species tend to rest in the forest rather than indoors. In December in the Kale Valley *A. minimus* rested in houses by day ; in May in the Kabaw Valley it did not. It is believed that where, as in the Kabaw Valley, forest comes up to the edge of the village, *A. minimus* seeks shelter for the day in the forest, but that where, as in the Kale Valley, the village stands in a clearing, it rests by day in the houses near where it has fed. The facts observed, however, do not preclude the possibility of a seasonal change in resting habit. It is clear that *A. minimus* is not invariably a house rester and the importance of catching by night to gain a true picture of the mosquito population is stressed.

(12) *A. hyrcanus* was usually the first species to appear after dark, followed by *A. philippinensis*, *A. leucosphyrus* and *A. minimus* in that order, the last named being usually most abundant in the second half of the night. Time of greatest activity was, however, variable in all species. There were only a few isolated instances of Anophelines biting in the forest by day.

(13) *A. hyrcanus* was attracted to light.

(14) Larvae identified as *A. pallidus* with the aid of the standard Indian keys invariably proved to be *A. philippinensis* on emergence.

(15) The variation found in *A. minimus* was considerable and is briefly discussed. The only constant difference between *A. minimus* and *A. aconitus* was found to be the flavescence on the proboscis.

References:

CHRISTOPHERS, S. R. (1933). The Fauna of British India, including Ceylon and Burma. Diptera, Vol. IV. Family Culicidae. Tribe Anophelini. xii, 371 pp., 3 pls. London, Taylor & Francis.

CLARK, R. H. P. & CHOWDHURY, M. A. (1941). Observations on *Anopheles leucosphyrus* in the Digboi area, Upper Assam.—J. Malar. Inst. India, **4,** pp. 103–107.

COVELL, G. (1944). Notes on the distribution, breeding habits, adult habits and relation to malaria of the Anopheline mosquitoes of India and the Far East.—J. Malar. Inst. India, **5,** pp. 399–434.

MCARTHUR, J. (1946). Malaria transmission in Borneo.—Lancet, 27th July 1946, p. 117.

——. (1947). The transmission of malaria in Borneo.—Trans. R. Soc. trop. Med. Hyg., **40,** pp. 537–558.

PURI, I. M. (1931). Larvae of Anopheline mosquitoes with full descriptions of those of the Indian species.—Indian med. Res. Mem., no. 21, vi, 227 pp.

——. (1938). Synoptic tables for the identification of the Anopheline mosquitoes of India.—Hlth Bull. no. 10 (Malar. Bur. no. 2), Delhi. 62 pp.

——. (1941). Synoptic tables for the identification of the full grown larvae of the Indian Anopheline mosquitoes.—Hlth Bull. no. 16 (Malar. Bur. no. 7), Delhi. 109 pp.

RAMSAY, C. G., CHANDRA, S. N. & LAMPRELL, B. A. (1936). A record of an investigation to determine the androphilic indices of certain Anopheline mosquitoes collected on tea estates in Assam and Northern Bengal.—Rec. Malar. Surv. India, **6,** pp. 49–52.

TOUMANOFF, C. (1936). L'Anophélisme en extrême-orient. Contribution faunistique et biologique. viii, 434 pp. Paris, Masson et Cie.

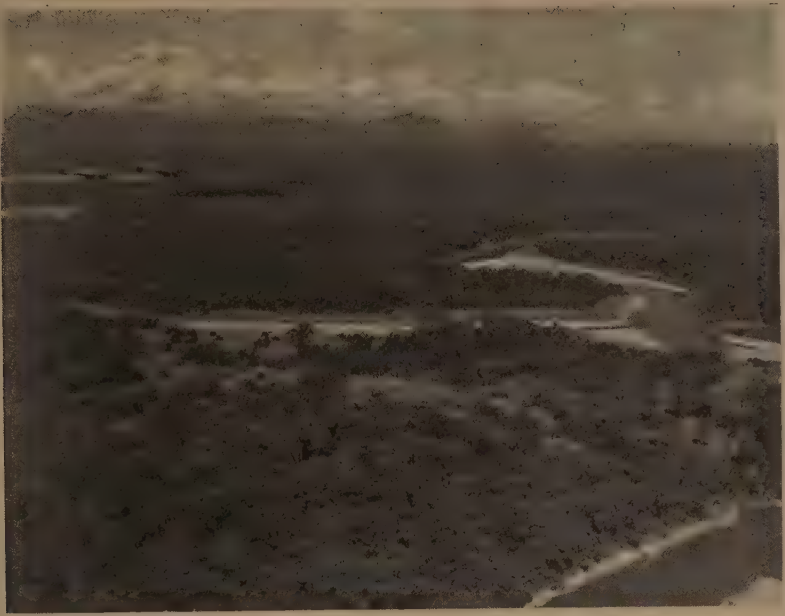

Fig. 1. The Kabaw Valley from the air.

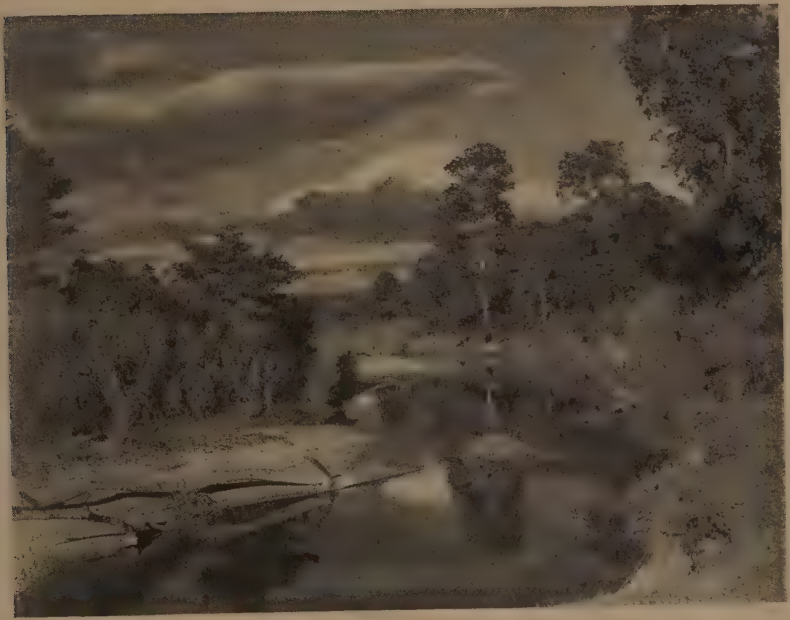

Fig. 2. A stream through the Kabaw Valley.

A METHOD OF ASSESSING THE ACARICIDAL PROPERTIES OF DDT AND "GAMMEXANE" PREPARATIONS IN FIELD TRIALS.*

By S. G. Wilson, B.Sc., Ph.D., M.R.C.V.S.,
Senior Veterinary Research Officer, Uganda.

For many years the control of East Coast Fever has depended on the eradication of the various tick-vectors by means of arsenical dips. Arsenic had many disadvantages which limited its usefulness but the recent demonstration of the acaricidal powers of DDT and "Gammexane" has improved the outlook for future control measures. Neither of these compounds is readily toxic to bovines. Their lethal action is quicker than arsenic and ticks once exposed to preparations of proper strength seldom recover and it is unlikely that any tick species will become resistant to both in the way species of *Boophilus* have become arsenic-resistant in South Africa. The residual effects of DDT and "Gammexane" preparations under tropical African conditions have not yet been fully determined but they are certainly greater than arsenical solutions. The fact that they lose their insecticidal powers when exposed to such conditions suggests that these compounds may be safe to use as sprays for cattle confined in crushes and they may, therefore, be cheaper to apply than arsenical solution which can only be used in properly constructed dips or enclosed sprays. Much preliminary experimental work has yet to be done, and the present paper describes a technique adapted at Entebbe to assess the acaricidal properties and residual effects of DDT and "Gammexane" applied to bovines at various dilutions by various methods.

Technique.

The distribution of the various species of ticks on the body of bovines has not yet been determined with the same exactitude as for *Ixodes ricinus* on sheep (Milne 1947). Numerous collections have been made from bovines over a period of several years in various localities (Wilson 1946) and it is evident that each species of tick has a definite preference for a particular site. The most frequent sites of attachment of the common species are shown in fig. 1.

In most localities, adults and nymphs of *Rhipicephalus appendiculatus*, Neum., attach themselves to the inner or concave surfaces of the ear auricula and especially along its anterior border. A few adults, seldom exceeding 10 per cent. of any infestation, are to be found round the base of the horns, eyelids, cheek and neck and on the tail switch. In certain areas of Uganda where infestations are very heavy and *R. appendiculatus* is the predominant species, numerous engorging adults can be collected from udders, scrotal region and flanks. Adults and nymphs of *Boophilus fallax*, Minning, and *B. decoloratus*, Koch, favour the belly and flanks. Adults of *Rhipicephalus evertsi*, Neum., are common on the anal region but the nymphs occur in clusters in the depressions on the inner ear surface at the base of the auricula. Adults and nymphs of *Amblyomma variegatum*, F., are most common on the udders, flanks, dewlap and brisket but larvae are found on the ears and head. Adults of *Rhipicephalus simus*, Koch, and *Hyalomma impressum*, Koch, are found together on tail switch and feet. The site of attachment of the larvae of most of these species has not been fully determined. A total count of all of them on a bovine before and after treatment with an acaricidal preparation would be most laborious even if the count was limited to adult ticks. Furthermore, as tick counts from different animals in any one herd show a wide range of variations, counts of single or small groups of animals would give a most unreliable estimate of the infestation rate. Owing to the

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[*] This paper is published with the permission of the Director of Veterinary Services, Uganda.

rough and woody nature of the pasturage and the fact that cattle are grazed over large unfenced areas it is impossible to estimate tick population by dragging blankets over pasturage. The present method of assessing the immediate toxic effect and subsequent residual action of DDT and "Gammexane" sprays was to count at frequent intervals the adults of only two species, *i.e.* *R. appendiculatus* adults attached on the ears and *A. variegatum* adults attached on the udder and belly region. These species were selected because they are prevalent in the area under experiment, are relatively easy to count and observe, and, as they are both important disease vectors, their control is important. When cattle were sprayed at weekly intervals, counts were made immediately before spraying, and on the 3rd day. If the tick population had definitely decreased on the treated bovine on the 3rd day as compared with that immediately before treatment and on untreated control cattle grazing on the same pasturage, a killing action by the sprayed preparation was assumed. To test the viability of ticks found alive and attached on the 3rd day, a bag was secured to the ears either by tapes tied around the base of the horns or by an elastoplastic bandage round the base of the ear. The detached ticks were collected from the bag on the 7th day and tested in the laboratory for viability ; males if unaffected should live for at least 3 days and engorged females should lay a normal number of viable eggs. To test the immediate toxic effects, ticks were counted every 24 hours for the first two days after spraying. Owing to practical difficulties, intensive observations on individual ticks were limited and confined to engorging females. The position of females found attached on the ear on the 3rd day was plotted and if these or other females were found engorged on subsequent days up to the 7th day it was assumed that the residual effects of the acaricidal sprays had been lost on or after the 3rd day. The amount of bleeding or excoriation along the anterior auricula border also gave some indication of the lethal effects of the sprays. Bleeding, inflammation and scab

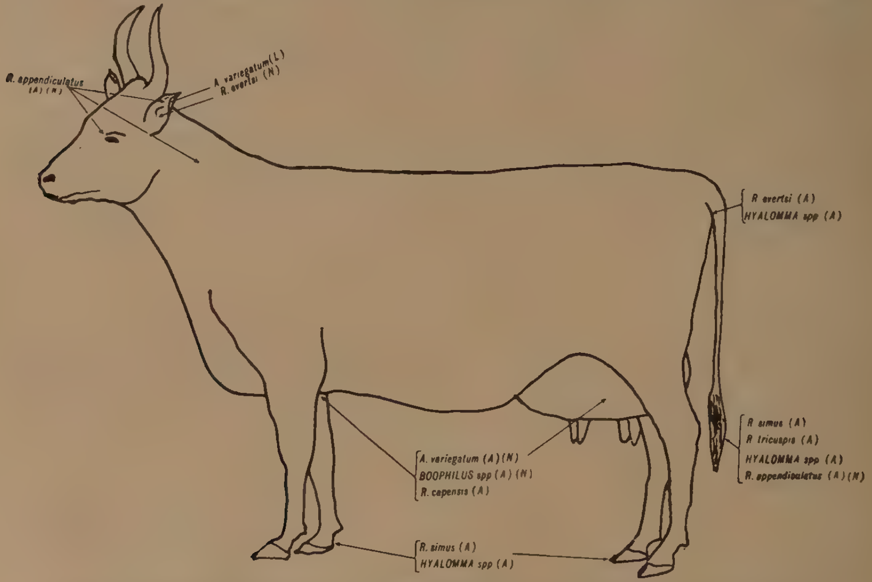

Fig. 1.—Diagram showing the most usual sites for attachment of the common tick species in Uganda.
A=Adult. N=Nymph. L=Larvae.

formation were evident along the border on unsprayed control animals where ticks remained alive to full engorgement, but if the spray was effective, and caused ticks to drop off prematurely, no inflammation was evident. No attempt was made to estimate the repellent effect of any of the preparations by counting the total number of ticks, alive and dead, shed by treated and untreated bovines.

Methods of Application of the Preparations.

Spraying was adopted when the quantity of the substance under test was limited and it was in the form of an emulsion or water dispersible powder. A large flit-gun was used in the earlier experiments but this is laborious if a large number of cattle have to be treated and, as the spray is intermittent, there was a strong tendency to spit. Measured quantities of fluid were applied to the ears of cattle by means of an Aerograph F.P. Model spray gun. This was fitted with a small container to hold fluid, air pressure being supplied by a Four-Oaks sprayer connected to the gun-butt by a reinforced rubber hose and the jet regulated to deliver 65 cc. per minute at 25 lbs. pressure per sq. inch. It was, however, difficult to maintain a steady working pressure and the cone of the spray and the amount delivered was too small for large scale experimental work.

A Four-Oaks Kent sprayer and a " Solo " double-action hand pump were used for body spraying. A " swirling-jet " nozzle (fig. 2) with a 1·5 mm. orifice was found to give a suitable type of coarse spray with a sufficiently large cone to deal expeditiously with numerous cattle in crushes.

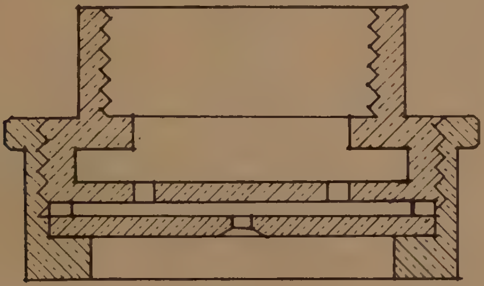

Fig. 2.—The " Swirling-jet " type of nozzle used with the Kent Sprayer.

A combined spraying-dipping method now in use may be applicable to field work in addition to its experimental value in testing limited quantities of test fluid against body and ear ticks. Cattle are forced to walk through a shallow bath, 4 feet deep and 10 feet long, containing the test fluid. In this way all ticks attached to the feet, legs and underside of the body (fig. 1) are brought into contact with the killing fluid, and as the cattle emerge up a narrow crush their head and ears are sprayed with a Kent or Solo sprayer. The Kent sprayer is replenished with fluid from the bath but the end of the rubber-hose attached to the " Solo " pump may be immersed in the bath and cattle sprayed direct with the bath fluid. In a small bath such as this the fluid can be renewed at frequent intervals and as the area sprayed is small the treatment is likely to be more thorough with unskilled labour than if the whole body was sprayed.

Preliminary Experiments with DDT and " Gammexane " Sprays.

In the first experiment, begun in September 1946, a DDT preparation (A.19) containing 23 per cent. DDT. (80 per cent. p.p′) was used as an emulsified solution with a concentration of 5 per cent. DDT. Six animals were divided into two groups; the ears of the four calves in Group I, Nos. 5392, 5465, 5467 and 5416, were treated by means of a large flit-gun with 25 to 30 cc. of this spray solution immediately before they entered a tick-infested paddock and again on the 9th day, while the two calves in Group II, Nos. 5562 and 5583, were given a single application of 5–10 cc. of the same spray on the 2nd day. The adult *R. appendiculatus* present on both ears only were counted daily until the 20th day, with the results given in Table I. Both groups of calves were thoroughly sprayed on the 20th day and removed from the tick-infested paddock.

TABLE I.

Average daily number of live ticks on the ears of each animal.

Day of Experiment	Average No. of live ticks per ear	
	Group I	Group II
1st	2·2 (S)	7·0
2nd	1·2	6·2 (S)
3rd	1·0	3·5
4th	0·9	2·0
5th	1·0	2·5
6th	1·0	2·5
7th	1·0	2·5
8th	1·5	2·7
9th	1·7 (S)	2·5
10th	0·75	5·3
11th	0·5	6·6
12th	Nil	7·0
13th	Nil	7·7
14th	0·5	8·0
15th	0·25	8·0
16th	0·75	9·3
17th	1·15	7·0
18th	2·0	6·5
19th	4·5	6·5
20th	6·0	5·0

S=Sprayed.

The first day of the experiment was taken as 24 hours after exposure and the results were interpreted as follows. A gradual decrease in the number of attached live ticks occurred until the 4th day, the ticks alive on any one day had either dropped off or were still attached but dead 24 hours later. An increasing number of ticks attaching after the fourth day lived and on the 9th day engorged females were found which must have been attached for 3 or 4 days. One engorged female collected on this day oviposited viable eggs that produced healthy larvae. The second application of spray on the 9th day gave similar results, *i.e.* the ticks attached at the time of spraying and those attempting to feed on the days immediately subsequent were killed, but on the 14th and 15th day, or 5 and 6 days after spraying, ticks were attaching and remaining alive and females were almost fully engorged by the 20th day.

In Group II the application of a small quantity of the spray to the inside of the ears caused some reduction of live ticks found on ears for about 7 days, but some engorging females remained attached and apparently unaffected. Tick infestation of the ears was relatively high from the 10th day onwards.

From these preliminary observations it was obvious that a 5 per cent. DDT spray will kill R. appendiculatus *but the effect soon wears off and cattle must be sprayed every 7 days to prevent nymphs engorging and falling off to moult and also to prevent females engorging and falling off to oviposit and continue the life-cycle.* Engorging females were more resistant to the effects of DDT than the males.

In October 1946 a second experiment was carried out. Nine cattle were herded in the same tick-infested paddock as in the previous experiment. Three bovines, No. 5393, 5464 and 5566, were sprayed with 2·5 per cent. DDT emulsion (A.19), three others, Nos. 5437, 5469 and 5575, with a suspension containing 10 per cent. Gammexane dispersible powder P.530 giving 0·65 per cent. active gamma isomer, whilst three, Nos. 5395, 5478 and 5573, were used as controls. The six cattle in the treated groups were sprayed on the 1st and 7th days and, as in the previous experiment, only the ears were sprayed and ticks were only counted on these sites. These counts were low as some of the ticks had been killed by the DDT spraying in the previous experiment and others had engorged, therefore the *total* live ticks found on each group are given in Table II and not the *average* number per animal as in Table I.

TABLE II.

Total ticks counted daily on the ears of each group of three animals.

Days	Total ticks on 3 Bovines sprayed with DDT	Total ticks on 3 animals sprayed with "Gammexane"	Total ticks on 3 control animals
1	Few dead ticks	Nil. Few dead ticks ...	4♂♂ 2♀♀
2	,, ,, ...	,, ,, ,, ...	2♂♂ 2♀♀
3	,, ,, ...	,, ,, ,, ...	1♂ 1♀ 1 N.
4	1♀ Few N. L.	Few N. L.	1♀ Few N.L.
5	,, ,, ...	,, ...	,, ,,
6	3♂♂ 1♀ N.+L.+ ...	1♀ N.+L.+	1♂ 1♀ N.+L.+
7	3♂♂ N.+L.+	,, ,, ...	,, ,,
8	Dead ticks only	Dead ticks	2♂♂ 1♀ ,,
9	,, ,, ...	,, ,, ...	3♂♂ 2♀♀ ,,
10	1 N	1 N. 1 L.	3♂♂ 2♀♀ ,,
11	1♀ 2 N.	4♂♂ 1♀ 1 N.	2♂♂ 2♀♀ ,,
12	1♂ 1♀ 4 N.	4♂♂ 2♀♀ Few N. L. ...	3♂♂ 3♀♀ ,,
13	1♂ N.L.+	3♂♂ 3♀♀ N.+L.+ ...	4♂♂ 6♀♀ ,,
14	1♀ N. L.+	3♂♂ 1♀ N.+L.+ ...	5♂♂ 6♀♀ ,,

N=Nymphs chiefly *R. appendiculatus* and *R. evertsi*.
L=Larvae chiefly *A. variegatum*.
Few=1—5 in number.
+=> 5 in number.

In both the DDT and "Gammexane" groups, larvae, nymphs and adult ticks remained on the ear and engorged after the 4th day and, as in the previous experiment, it was necessary to spray the animals every 7th day in order to interrupt the life-cycle of developing ticks.

Fields Experiments with DDT and "Gammexane" Sprays.

Cattle, from African-owned herds, infested with various tick species, were sprayed with DDT and "Gammexane" solutions under field conditions. Two dilutions of the DDT emulsified solution (A.19) containing 5 and 2·5 per cent. of the para-para isomer respectively and three dilutions of the "Gammexane" dispersible powder (P. 530) containing 0·65, 0·065 and 0·325* per cent. of the active gamma isomer were used. These preparations were applied with the Aerograph F.P. Model spray gun. Groups of four calves, about 2 years old, were treated in each case and a further group of four was set aside as a control. The ears of the animals were sprayed once a week for 14 weeks except on the 12th week when they were treated on the 1st and 4th day of the week. Ticks were counted each week on the day of application and as often as possible on the 4th day. The results with the DDT sprays on *R. appendiculatus* are shown in fig. 3.

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[*] 10 gms., 1 gm., and 5 gms. of P.530 powder respectively containing 6·5 per cent. gamma in 100 gms. of water.

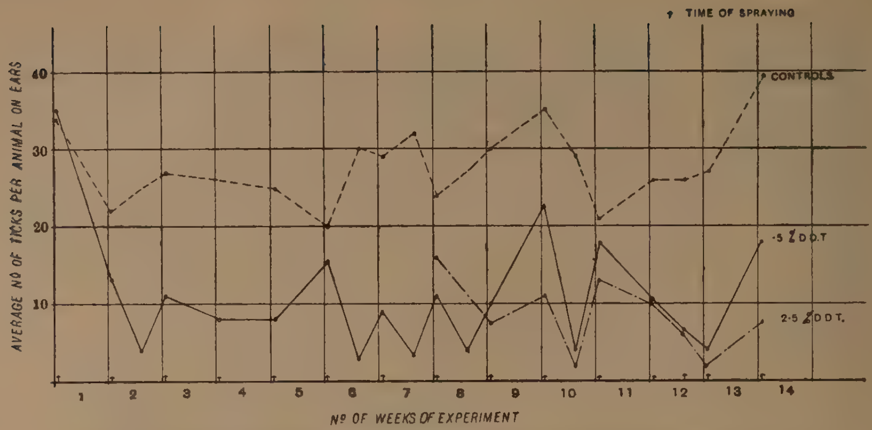

Fig. 3.—Tick counts on ears of animals sprayed with 5 per cent. and 2·5 per cent. DDT emulsified solution compared with a control group.

The number of adult *R. appendiculatus* per animal on the ears of the control cattle varied between 20 and 40. The ticks on the ears of those animals sprayed with 5 per cent. DDT emulsified solution at the time of spraying invariably died within 24 hours but when recounts were made on the 4th day a new " crop " were attaching and the numbers of ticks attached increased until the 7th day, when the cattle were resprayed, confirming the conclusions reached in the previous laboratory experiment. Where mid-week counts were made, only on one occasion was the number of ticks attached lower on the 1st day of the week than at the previous mid-week counting and this was in the 13th week following the twice-weekly spraying of the 12th week. Consequently with the present DDT formulation cattle should be sprayed twice a week during the early weeks of a tick eradication campaign. On the 8th week of the experiment, four additional calves were included and sprayed with 2·5 per cent. DDT, and the tick control was usually better than that afforded by 5 per cent.

The results with the " Gammexane " solution on *R. appendiculatus* are given in fig. 4.

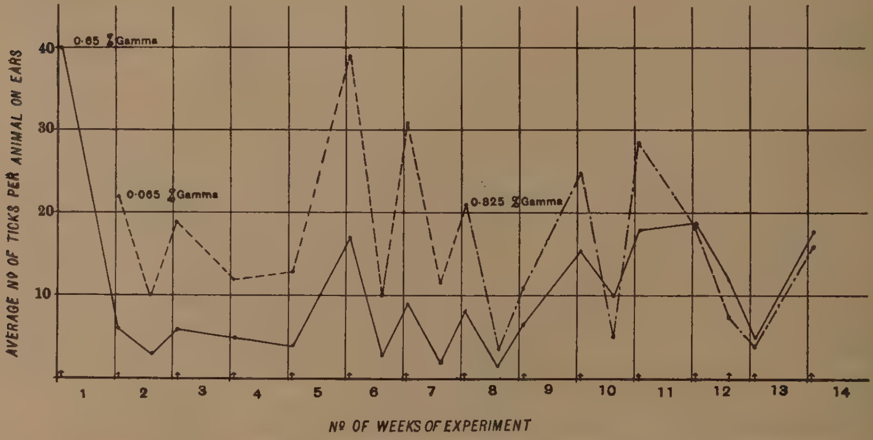

Fig. 4.—Tick counts on ears of animals sprayed with three concentrations of " Gammexane ".

The control animals were the same as in the DDT group. Using a 0·65 per cent. gamma solution, the tick control was good but again the residual effect was short, and from the 4th day after treatment ticks attached and remained alive to engorge; it was as effective as a 5 per cent. DDT spray, *i.e.*, an effective ratio of 1 : 6. The 0·065 per cent. gammexane solution was soon discarded as not sufficiently toxic against *R. appendiculatus* to merit further consideration. From the 8th week onwards the same four cattle were sprayed with 0·325 per cent. solution and this was considered as effective as the 0·65 per cent.

A final experiment was carried out over a period of 3 weeks to test the effects of adding coumarone resin to the DDT emulsion. Half a pound of resin was added to half a gallon of a 25 per cent. A.19 emulsion and this stock was later diluted with water to contain 2·5 per cent. DDT and applied to the ears of calves with the Aerograph spray gun. The results of the coumarone-DDT and simple DDT sprays are given in fig. 5.

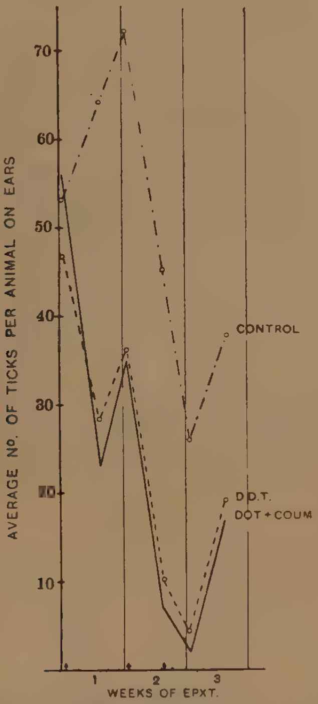

Fig. 5.—Tick counts on ears of animals sprayed with a 2·5 per cent. DDT emulsified solution with and without the addition of coumarone resin compared with control animals.

Each group consisted of eight cattle and only the ears were sprayed. There was no difference whatever between the two sprays, *i.e.*, the coumarone resin did not increase the residual effects of the spray at this dilution. The general trend of the tick population is very similar to that shown in figs. 3 and 4. After treatment the number of ticks decreased until the 5th day but rose again towards the end of the week. In the 2nd week after two applications the number of ticks remained low until the middle of the 3rd week, *i.e.*, after 7 days the numbers of ticks attaching and surviving on the ears were increasing. The decrease in ticks during the second week was also partially due to an unexpected change of grazing grounds from heavily tick-infested pastures to more lightly infested areas and a similar trend is shown in the control group of animals.

Summary.

DDT and "Gammexane" have many advantages over arsenic as acaricidal agents.

The sites of attachment of the common tick species on bovines are recorded and the killing properties of various dilutions of DDT and "Gammexane" are assessed by observations on *R. appendiculatus* attached on the ears and *A. variegatum* attached on the udder and flanks of cattle.

The preparations were usually applied as sprays by means of a flit-gun, an Aerograph spray gun, and Kent sprayer or "Solo" hand pump. A "swirling-jet" nozzle was found most suitable. A possible combined spraying-dipping method using a shallow bath is also described.

In preliminary experiments, a spray containing 2·5 per cent. DDT and a preparation containing 1 : 10 of the "Gammexane" dispersible powder P.530 killed all ticks attached at time of spraying but the residual effects were lost about the 4th day after spraying. Field experiments gave similar results, but 1 : 20 of the P.530 powder (0·325 per cent. active gamma) proved equal in killing value to the 1 : 10 preparation.

The addition of coumarone resin did not increase the residual effects of a 2·5 per cent. DDT emulsified solution.

References.

Milne, A. (1947). The ecology of the Sheep Tick, *Ixodes ricinus* L.—Parasitology, **38,** pp. 34–50.

Wilson, S. G. (1946). Seasonal occurrence of Ixodidae on cattle in Northern Province, Nyasaland.—Parasitology, **37,** pp. 118–125.

A BIOLOGICAL TEST FOR ASSESSING THE ACARICIDAL PROPERTIES OF DDT AND "GAMMEXANE" PREPARATIONS.*

By S. G. LAWS.
Veterinary Research Laboratory, Entebbe, Uganda.

When DDT and "Gammexane" were first used in Uganda for work on tick control, the absence of a rapid and simple chemical test made it necessary to evolve a biological method which would assess the killing power of the preparations in use. Such a method, if it is to be employed to assess the results of field work, should satisfy the following conditions ;

(*a*) ticks should receive an initial wetting with the preparation comparable to that received when cattle are dipped or sprayed, and

(*b*) remain on a moist surface for a few hours, and then for a longer period in contact with the dried deposit, as in the case of the animal after it has been dipped or sprayed.

Preliminary Trials.

Ticks (*Rhipicephalus appendiculatus*, Neum.) were first exposed to impregnated filter paper in petri dishes, but this proved impracticable as larvae and even adults were liable to escape. Corked test-tubes, lined with impregnated filter paper, also proved unsuccessful for the filter paper remained moist for several days and many of the control ticks did not survive. When the corks were replaced with cotton wool, ticks escaped contact on the impregnated filter paper by attaching themselves to the plugs. The following method was finally adopted.

Apparatus required for each Test.

Two open-ended glass tubes 10 cm. long by approximately 2 cm. in diameter ; one pipette to deliver 1 ml. ; two pieces of thin white filter paper (Green's No. 401) 10 cm. by 7·5 cm. ; two strips of gummed paper (as used for sealing packages) 10 cm. long by 1 cm. wide.

Technique (fig. 1).

The particulars of the sample are written on the long edge of a piece of filter paper with indelible pencil, which is then turned over, rolled on a glass rod and inserted into the tube as shown in the figure. The sample is shaken to obtain an even suspension of any deposit and 1 ml. of the acaricide under test is distributed evenly over the surface of the paper by pipetting 0·5 ml. into each end of the tube. This quantity is sufficient to moisten the size of paper specified, but larger tubes and papers, and a correspondingly larger quantity of the sample, could be used. (During the first trials with this method, 50 ml. "Holborn" syringe barrels were used.) The second paper is inserted into the second tube and moistened with a similar quantity of the sample and the moistened paper is then slipped out of the tube and divided into two portions for use as end covers. One end of the tube containing the first test paper is closed with a portion of the second test paper *while still moist*, and the overlap firmly bound in position with a strip of gummed paper in the manner shown in the figure. This forms a close-fitting cap, which, when dry, can be slipped off and on very easily for examination of the ticks during the test. The tube, open end upwards, is stood on a glass plate on the bench.

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The ticks used in the test may be laboratory bred or collected from clean animals and all species and stages may be used but active adult males give quicker results. A piece of filter paper is placed in a petri dish and thoroughly saturated with the sample. The selected ticks are placed on the paper and, by means of a pladget of cotton wool well saturated with the sample, a firm but even pressure is applied. This wetting temporarily immobilises the tick and it may be necessary to remove it from the wool by means of a wooden toothpick. Ten ticks are treated in a similar manner and dropped one by one into the prepared tube. This stage is meant to resemble the wetting received by the ticks when cattle are dipped or sprayed. The end of the tube is closed with the other portion of the second test paper and both ends of the tube are thus sealed; thereafter the ticks are continuously exposed to the impregnated filter paper which remains moist for from three to six hours at room temperature. A control tube is prepared each time the test is performed, by substituting distilled water for the sample. The tubes are stored in a horizontal position and observed at 6, 24, 48, and 72 hours, to record mortality. Most if not all of the ticks in the control tube should remain alive and active at 72 hours, while mortality in the test will commence from the 6th to 48th hour, depending upon the killing power of the sample.

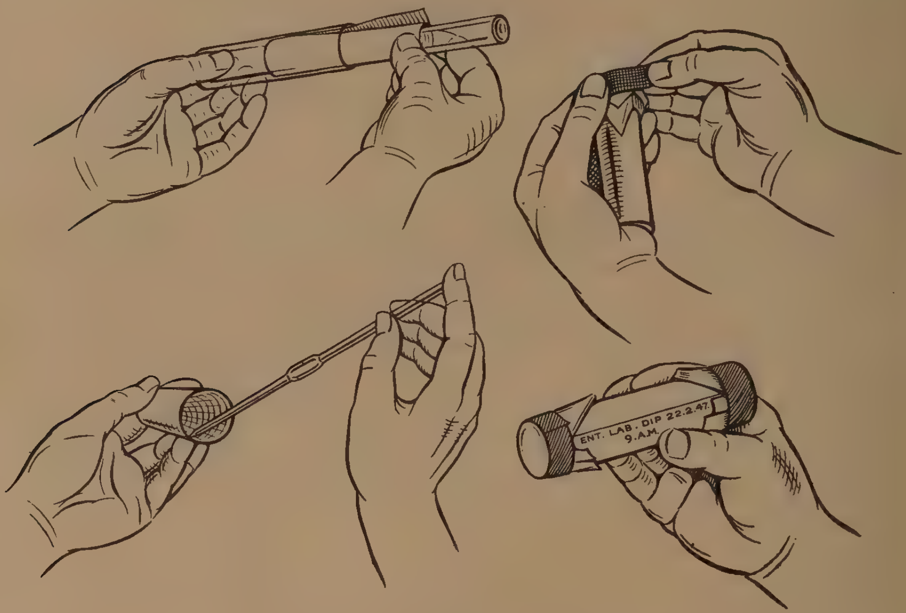
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Fig. 1.

Cleaning of Glassware.

All glassware is cleaned after use by immersion in three changes of power kerosene, 24 hours in each change, and finally boiled in soap solution to remove all traces of the insecticide.

Examples of a few selected tests.

Day of test	Sample	Ticks used	Observations			
			6 hours	24 hours	48 hours	72 hours
6.11.46	DDT 5 per cent. p.p′.	*R. appendiculatus*, 10 ♂♂	Alive, but sluggish	All dead	—	—
6.11.46	B.H.C. 0·65 per cent. gamma content	10 ♂♂	Sluggish	All dead	—	—
6.11.46	B.H.C. 0·65 per cent. gamma content (23 days old)	10 ♂♂	7 alive 3 dead	2 alive 7 dead 1 moribund	All dead	—
6.11.46	Arsenical Dip (0·08 per cent.)	10 ♂♂	All alive	7 alive 3 moribund	3 alive 7 moribund	All dead
6.11.46	Control (H_2O)	10 ♂♂	All alive	Alive and active	Alive and active	All alive and active

Notes.—A DDT emulsion, A.19, containing 23 per cent. DDT (80 per cent. p.p′.) was diluted with water to give a solution containing 5 per cent. of the active p.p′. isomer.

A benzene hexachloride dispensible powder, P.530, with a gamma isomer content of 6·5 per cent. was diluted 1 in 10 with water to give a suspension of 0·65 per cent. gamma content.

Acknowledgement.

I have to thank Dr. S. G. Wilson, Senior Veterinary Research Officer, Uganda, for the opportunity to evolve this method and for samples of the various preparations used in his experiments.

SUCCESSFUL METHODS OF TRAPPING *TROMBICULA* (ACARINA) WITH NOTES ON REARING *T. DELIENSIS*, WALCH.

By K. L. Cockings.

The importance in medical research of methods whereby mites of the family Trombiculidae can be trapped in large numbers needs no emphasis.

These mites are widely known in many countries of the world. In some, they are pests causing only irritation of the skin, as for instance the English " harvest-mite " (*Trombicula autumnalis*, Shaw, 1790) which in its larval form is often a nuisance to field workers during summer and autumn.

In other countries, as those in South East Asia and the Pacific area, certain species of *Trombicula*, namely *T. akamushi*, Brumpt, and *T. deliensis*, Walch, 1922, are proved to be vectors of the disease known as scrub typhus (Tsutsugamushi disease).

During 1944–45 I served as a member of the Medical Research Council's Typhus Team attached to the Scrub Typhus Research Laboratory, South East Asia Command, which was based at Imphal, Assam. Here, I spent some months studying and rearing *T. deliensis* and other Trombiculid mites.

Later, in England, I had the opportunity of working on *T. autumnalis* at and near Compton, Berkshire. The main object at this time was to evolve and test methods of trapping which might facilitate future investigations into the problems of scrub typhus abroad. In view of the morphological similarity of the known scrub-typhus vectors and *T. autumnalis*, it was considered probable that their reactions to warmth and light would also be similar ; and that methods of trapping based on observation of their natural tendencies could be universally used. In this paper, therefore, an account is presented of the methods of trapping (used at Compton) which are based on what would appear to be the characteristic behaviour of Trombiculid larvae. I also include a successful method, first described by Japanese workers, of recovering adult *Trombicula* from the soil ; and, separately, an account of my methods and techniques of rearing mites which were adopted whilst working at the Scrub Typhus Laboratory in Assam.

Brief Description of the Life-history of *Trombicula*.

Mites of the genus *Trombicula* attack man and other mammals during the larval stage of their life-cycle only. As a six-legged larva the mite emerges from an egg laid in or on the surface of the soil. It looks like a minute red spider approximately 0·4 mm. in length. To further its development, it is necessary for the larva to attach itself to the skin, and feed on lymph or tissue fluids for varying lengths of time according to species. Having become fully engorged, the larva drops from the host to the soil where, under favourable humid conditions, it changes into a nymph. The nymph (approximately 1 mm. in length) and the adult (2 or 3 mm.) live and feed in the soil. They have eight legs and their hairy bodies (which are to an extent hydrophobe) are constricted about the thorax and resemble a figure 8. Their colour ranges from " off white " to red.

In the past, investigations into important aspects of the scrub typhus disease have been restricted because of difficulty in obtaining large numbers of live, unengorged larvae from the field.

Williams (1946) describes a method of collecting free-living larvae of *Eutrombicula alfreddugesi* (Oudemans) by which the larvae are sucked into an aspirator as they appear on white saucers placed on the earth. Collections of larvae from boots and clothing have been described by other investigators (McCulloch), but as certain species of *Trombicula* appear to attack man more readily than others, the success of collections by this method would appear to be dependent on the species present. However, both methods are only suitable where there is an unusually high infestation.

It is hoped that the field mite-trap described in this paper will simplify, as it will undoubtedly increase, collections from areas where the larval population is low.

The following laboratory observations, which are relevant to the methods and results of mite trapping described herein, were made whilst rearing *T. deliensis* in Assam, 1945, and confirmed during recent work on *T. autumnalis* in Compton, 1946.

Observing the larvae of *Trombicula* under laboratory conditions one finds that they were very sensitive to changes in temperature. They are activated into brisk movement when warmed and, like other Arthropods, they become less active and eventually akinetic as the temperature is decreased.

They also show a strong tendency to climb upward even when they are within a completely darkened tube.

If enclosed in loose soil within a tube the unengorged larvae are shortly observed to appear on the surface of the soil, and within the uppermost portion of the tube.

Field Mite-trap.

The field mite-trap, constructed as described below, was used to trap unengorged larvae of *T. autumnalis* on chalk-down (short vegetation) near Compton, Berkshire. It is so designed that use is made of the natural tendency for the larvae to move towards light and to climb upward.

The trap is constructed as a thin metal tray, three feet square, having sides three inches deep. A hole ($2\frac{1}{4}$ in. diameter) is cut in the centre of the tray and the inside of the tray is painted dull black.

For use, the tray is inverted and placed on the earth to form a cover three inches high (fig. 1). To hold the top of the cover rigid two $1\frac{1}{2} \times \frac{3}{4}$ in. wood battens are used ; these are shaped so that when screwed to the metal the top of the cover becomes convex ($\frac{1}{2}$ in. rise).

A two-inch diameter specimen tube is inverted and fixed tightly into the hole with the aid of a thin rubber washer. (This central tube is the only source of daylight beneath the cover when the trap is set.) A small petri dish containing a fitted filter paper cone is placed on the earth directly beneath the outer fixed tube. Contact between the outer fixed tube and the filter paper cone is obtained by using a second specimen tube ($1\frac{1}{2}$ in. diameter). The closed end of this " contact tube " is made to slide freely within the outer fixed tube by means of a grooved rubber ring (Tube Plan fig. 1). The open end of the contact tube rests on the apex of the cone (Section, fig. 1). A small inspection hole made in the cover about nine inches from the centre enables one to see the internal tube arrangement and thus ensure a perfect contact. This is kept corked during the period of trapping.

A method of setting the trap is as follows : Firstly, the petri dish containing the filter paper cone is placed on the earth at the spot judged to be the centre of the one square yard which is to be trapped. The metal cover (without tubes) is then placed in position and the sides pressed firmly into the soil surface. The outer tube, containing the sliding " contact-tube ", is then fixed tightly into the hole in the metal cover. Contact is assured, if the " contact-tube " is sliding freely, by flicking or tapping the outer tube thereby causing the " contact-tube " to descend and to rest on the apex of the cone. To prevent desiccation of the trapped larvae, it is advisable to moisten the filter paper cone and to have some condensation within the tubes.

Apart from the degree of efficiency obtained, the advantage of using a trap of this design is obvious in that larvae which are on the earth or grass within one square yard are concentrated and trapped within a very small compass. The larvae go towards the source of light and climb the petri dish and the cone. On reaching the open end of the " contact-tube " they may squeeze between the rough surface of the filter paper and the glass, going inside the tube, or they may climb the outside of the " contact-tube " and pass by way of the grooves in the rubber ring into the outer fixed tube. The greater proportion of larvae take the latter, easier, course, providing that the cone is kept moist but not wet. When the cone is wet a large majority of the trapped larvae are found to be partially immobilised on the cone.

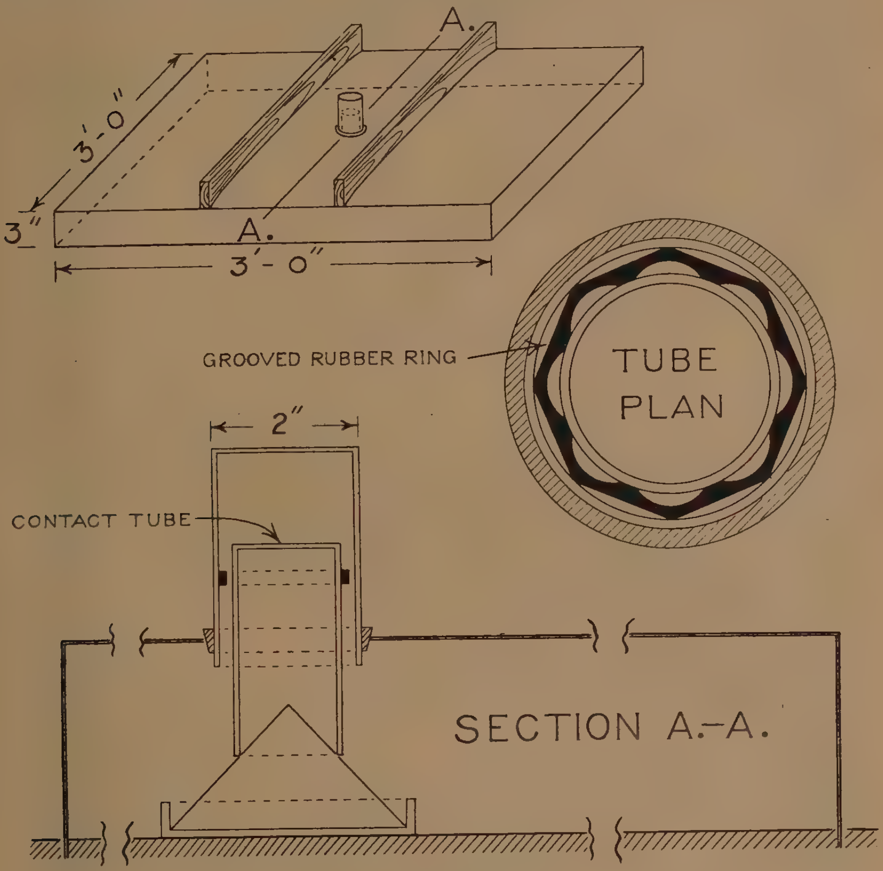


Fig. 1.—Field Mite-trap.

In so much as the larva is stimulated into increased activity when the temperature of its environment is raised, an added advantage in the trap's use is suggested by the observation that temperatures 10°F. higher than the outside air temperatures were commonly recorded beneath the metal cover. This increase was noted near Compton on days when there had been little direct sunshine. On one warm, sunny occasion the temperature beneath the cover was 30°F. higher than that of the outside air.

This general increase in temperature was attributed to the fact that the outside of the cover was also painted dull black, causing it to act as a black body. (For use in tropical climates this may not be advantageous.) Although it was also observed that numbers caught in the field mite-trap during 4½-hourly periods of indirect sunlight were comparable with those caught over similar periods of direct sunlight, it is suggested that the optimum trapping time is reduced by trapping under the latter condition.

In order to obtain a representative number of larvae, a trapping period of 4½ to 5 hours under warm, dry conditions is considered to be the most economical. On three occasions the number of *T. autumnalis* larvae caught after 4½ hours was increased by less than 10 per cent. by leaving the trap in the same position for a further 19 hours.

The maximum number of larvae caught per square yard (to which other catches approximate) during any period lasting 4½ hours (sunlight) was 154. From a nearby plot which was trapped at a later date, 172 larvae was the maximum number caught during any period of twenty-four hours.

The following results suggest that a high degree of efficiency is obtained when the trap is used on chalk-down. (The term " chalk-down " refers to a locality where soil to a depth of only a few inches covers pure chalk and bears short grass, a variety of flowers, and low bushes of gorse and juniper.)

Within the small area shown in fig. 4 an average of four catches exceeded 100 larvae. On each of these occasions the exact area, over which the trap had been set for twenty-four hours, was immediately occupied by bait-animals which proved acceptable hosts for *T. autumnalis* (2 guineapigs and 2 golden hamsters, *Cricetus auratus*). These were placed in a specially constructed frame (see trapping method 2) for a period of 4½ hours. On examination, a maximum number of three larvae was found attached to any one batch of four animals, despite the fact that the conditions to which the animals were exposed were considered ideal for bait-animal trapping.

No other experiment was conducted to determine the efficiency of the trap on chalk-down, but in the grounds of the A.R.C. Field Station, Compton, it was set on a lawn (close grass, three to four inches high) for twenty-four hours (10 a.m. to 10 a.m.). No larvae were trapped during this period. Fifty unengorged larvae adhering to wet filter paper were placed under the cover approximately twenty-one inches from the centre. The trap was then reset in its original position. Four and a half hours later 43 larvae were recovered from the tubes.

Bait Animals.

A preliminary experiment, repeated once, determined the choice of the guineapig and the golden hamster as the two most suitable animals available for collecting larvae in the field. Other laboratory animals tested were the rabbit, white mouse and Orkney vole (*Microtus orcadensis*).

The choice was based on numbers of larvae found attached to the animals after they had been exposed to similar conditions for the same length of time. All-wire cages, each containing two animals, were placed on the earth to form a circle within a heavily infested area. A space equivalent to the width of a cage was left between each and the cages were moved one space hourly until a circuit had been completed.

The examination of the animals following each experiment showed the guineapig to be the most effective host. The second highest number of larvae was found on the golden hamster, which was followed in order of efficiency by the Orkney vole, rabbit and the white mouse.

In order to compare numbers of larvae collected from bait-animals with those caught in the field mite-trap, a frame was constructed which confined the animals within a similar area to that covered by the trap. As shown in fig. 2 the frame is made so that the lower edges of the wooden sides project below the wire mesh bottom. This projection is bevelled to facilitate pressing that part into the soil surface, thus isolating the larvae within the enclosure from those outside. As a precaution against the possibility of larvae from outside the frame being attracted by movements of the animals and gaining access by climbing the wooden sides, the outer surface of the wood is impregnated with dibutyl phthalate, which is known to be rapidly lethal to these larvae.

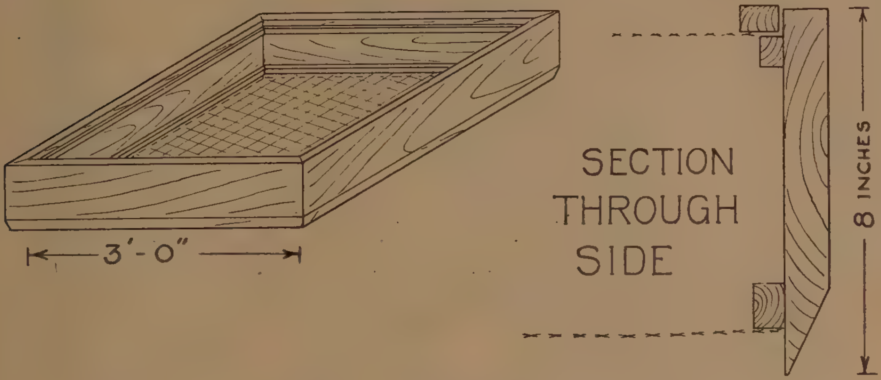


Fig. 2.—Animal Field Frame.

Throughout all investigations concerning the comparative efficiency of bait animals and the field mite-trap, two guineapigs and two golden hamsters were put together in one frame.

Numbers of larvae picked up by animals and those caught during the same period in the trap were felt to be comparable only on occasions when the minimum air temperature exceeded 55°F. ; for example—during three trapping periods of four and a half hours, when the air temperature ranged between 40° and 50°F., the number of larvae picked up by animals was less than half the number caught in the trap. This was probably due to the raised temperatures under the trap which ranged between 60° and 65°F.

Following one of the three trapping periods, the area previously occupied by animals was immediately covered by the trap. This resulted in a further collection of forty larvae after twenty-four hours, which raised the total catch for that plot to 10 per cent. higher than the adjacent field mite-trap catch of four and a half hours' duration.

On five separate occasions bait-animals were put down for periods ranging from four and a half to five hours. Larvae found attached to these after each period exceeded 110 (from one square yard). The minimum air temperature to which the animals were exposed was 55°F. and the maximum 75°F. Over each of these plots, immediately following the animals, the field mite-trap was set for twenty-four hours ; this resulted in a maximum additional catch of only fifteen larvae per square yard.

In the laboratory at Compton observations were made on the distribution of larvae on the bait-animals. Over 70 per cent. of all larvae found attached to guineapigs were found grouped around the genital area, particularly near the anus. Approximately 20 per cent. were found attached to the soft skin of the perineum, and less than 10 per cent. in the ears.

Larvae attached to golden hamsters were more evenly distributed between these areas. Very few larvae were found on any other part of the body in either case.

The duration of the feeding time was suggested when repeated observations on ten guineapigs showed that over 80 per cent. of the larvae left the animal between thirty and sixty hours after attachment. In one instance the number of larvae counted on two guineapigs, forty-eight hours after attachment, exceeded 110 ; all of these were found to have fed and dropped from the animals three hours later.

All larva-infested animals connected with field experiments were anaesthetised in the laboratory using Bromethol (aqs. sol.) 1 in 40, which was injected intraperitoneally. For a guineapig of moderate weight 1 c.c. was found sufficient to anaesthetise the animal for a few minutes only. Hamsters injected with ½ c.c. remained anaesthetised for approximately ten minutes.

The operation of injecting the animals was simplified by the use of a method which was adopted at the Scrub Typhus Research Laboratory, Imphal, Assam, in 1945. This involves the use of a small mesh wire cylinder, closed at one end by a cotton wool plug, into which the animal is just able to squeeze. A rectangular hole cut in the wire is covered on the outside by a detachable wire door. (When handling wild rats in Assam, a cloth funnel also was used ; the small opening of the funnel being attached to the open end of the wire cylinder ; the large opening to the rats' cage.)

Most wild or semi-wild small animals appear eager to squeeze themselves into the wire cylinder, where they seem to feel secure especially if the interior is darkened by a slip-on cover of some kind.

If the wire cylinder is held horizontally with the door downward, the animal is secured (using a rolled cloth bung) in such a position that on removal of the door the perineum is exposed. Once secure it could then be turned upside down and the Bromethol injected. The whole operation was usually completed within one and a half minutes.

Before exposure to infested ground the hair of each animal was clipped short around the genitals, perineum and ears, to facilitate rapid examination.

I am indebted to Dr. W. S. Richards for his idea that a " universal " arm should be made (similar to Angle-poise lamp system) which would bear the weight of a dissecting microscope head. Such an arm was made and proved invaluable in the examination of an animal from all angles under the microscope. A thorough examination and larva count could be made in less than four minutes.

Laboratory Light-trap.

The laboratory light-trap was originally devised and proved efficient at the Scrub Typhus Research Laboratory, Assam, 1945, following experiments conducted by Major H. M. Thomas, R.A.M.C., which showed that the larvae of *Trombicula deliensis* moved towards light. The trap was primarily used in order that only replete larvae for rearing purposes might be recovered from live rats.

Hitherto the most popular methods of recovering replete larvae from animals have been similar to that by which the larvae are collected from a large white tray, having dropped from animals in a wire cage which is supported above the tray. An advantage of the light-trap is that the trapped larvae are collected from a small petri dish.

At Compton the light-trap method was again employed in recovering fully engorged larvae, this time of *Trombicula autumnalis*, from the bait animals.

Fully engorged larvae were also recovered from straw which had been used as bedding for the guineapigs and hamsters. Partially engorged larvae were obtained in large numbers from the severed ears of dead rats and rabbits, some unengorged from a " flagging-cloth " (see trapping method 4).

The component parts of the light-trap as shown in fig. 3 are as follows :—(*a*) a small wooden box with a wire-mesh end, (*b*) a black paper funnel nine to twelve inches long, (*c*) a filter paper tongue to be attached by wax, (*d*) a petri dish containing water, and (*e*) a 40-watt lamp or other dependable means of illumination. (The efficiency of the trap depends largely on the careful construction of the box.)

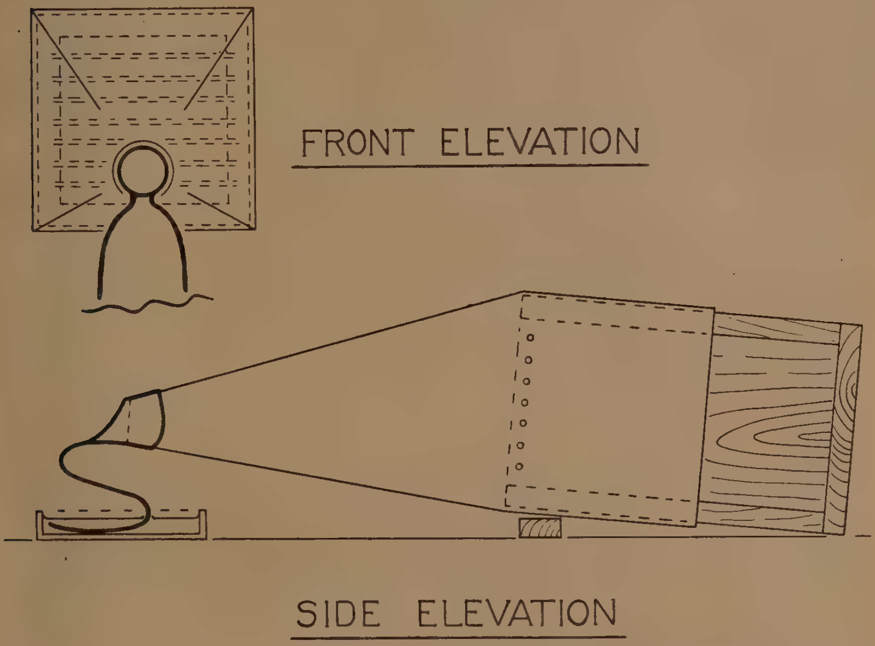


Fig. 3.—Laboratory Light-trap.

In order to house two guineapigs a 9×6×6 in. box was made from ½-inch thick wood ; the inner surfaces were smoothed to prevent obstruction to a walking larva. All joints were tightly fitting and light-proof. The whole of one end of the box was closed only by a stout wire-mesh door ; this was hinged at the top and fastened by means of a small hook in such a way that no wire protruded beyond the outside dimensions of the box. A black paper funnel, twelve inches long was then made ; the large end of this was squared to fit over the " open " end of the box ; the small end having an aperture one inch in diameter. The funnel was strengthened by strips of adhesive paper. A filter paper tongue was then attached to the small end of the funnel and this, when the trap was set, dipped into a petri dish containing water. The open end of the trap was raised so that larvae would be walking up a slight gradient, and a 25- or 40-watt lamp was placed in line with the funnel, nine inches away.

To test the efficiency of the trap, fifty fully engorged larvae of *Trombicula autumnalis* were shaken into the box, and the trap set. Forty larvae were recovered within twenty-five minutes, and all of them within forty-three minutes.

Two guineapigs infested with over 100 larvae were put into the box. Larvae appeared in the trap and were periodically picked out and counted. When the number trapped exceeded 100 and no further larvae appeared for one hour, the animals were taken out of the box. A thorough examination of the box revealed only two larvae which were adhering to droppings ; none was found on the paper funnel.

On occasions, larvae were seen to appear on the filter paper tongue within three minutes of placing the funnel in position. It was therefore concluded that the trap was remarkably efficient for collecting engorged larvae.

When the light-trap was used to recover partially engorged larvae from the ears of dead animals, straw bedding, etc., the substitution of a specimen tube for the filter paper tongue was sometimes advantageous. The tube contained a drop of moisture and was attached to the end of the funnel by a strip of adhesive paper. A black paper cylinder enclosed all but the base of the tube. Over 2,000 mixed larvae were recovered from ears of dead rabbits in one night.

An observation made in Assam, 1945, that *T. deliensis* larvae tended to leave a dead animal in a certain order according to the degree of engorgement was confirmed during the recent work on *T. autumnalis*.

It was observed that the unattached and therefore unengorged larvae left almost immediately following the death of both rabbit and rat. The most fully engorged followed shortly afterwards, and larvae considered large enough to undergo metamorphosis left within three to four hours. The partially engorged larvae which had fed for only a short time before the animal's death remained *in situ* for twenty-four or more hours. It was therefore possible roughly to divide the larvae appearing in the trap into three sizes by changing the tube (or picking from the filter paper) at intervals suggested by this observation.

Combination of Laboratory Light-trap and Flagging.

A method known as flagging (i.e. trailing a coarse cloth over the ground), though popular for collecting ticks, has been discounted as a practicable means of collecting *Trombicula* larvae in large numbers. The outstanding disadvantages of this method are, firstly, that it is highly probable that only a small proportion of the *Trombicula* larvae present are picked up on the cloth. Secondly, the task of collecting these minute larvae from cloth has proved even more tedious than making collections from white saucers or boots but the light-trap can be used in such cases to concentrate the larvae from the cloth into a tube.

Towards the end of the season of larval activity (near Compton) when few specimens were found trapped in the field mite-trap and several examinations of boots revealed none, eight larvae were recovered from a weighted "flagging cloth" six inches wide, which was trailed over soil bearing short vegetation. The cloth was placed on a six-inch wide strip of small-mesh wire netting ; both were then rolled together to form roughly a coil six inches long and four to five inches in diameter. This was placed quickly into the box of the laboratory light-trap so that one end of the coil was exposed to the "open" end of the box, over which the black paper funnel was slipped. Although this is considered to be an inadequate method, unsuitable for accurate survey work, it is worthy of mention in that, in the absence of a field mite-trap, it may possibly be used to greater advantage than white saucer or boot collections where larvae are abundant.

It is also considered reasonable to assume that larvae may be recovered, in the field, from newly cut vegetation by a similar method ; using perhaps a large box open at one side to which a short metal funnel may be fitted. The degree of efficiency obtained would most probably depend on the rapid collection and enclosure of the vegetation. This, alone, could only be regarded as a "sampling method" as it is probable that the numbers obtained would not be representative of the number of larvae present on the earth, but it may be considered as a useful preliminary to trapping by the field mite-trap where the vegetation is high and a thorough collection of larvae is required.

Recovery of Adult *Trombicula* from Soil.

Near Compton, between July and early December 1946, over 750 adults of *Trombicula autumnalis* were recovered from the soil. The method employed was that of flotation which was first introduced by Japanese workers.

Soil samples were taken carefully with a spade, put into tins and transported six miles by truck to the laboratory. Each sample separately was sprinkled into a large wooden sink containing warm water to a depth of three inches. The soil was immediately stirred thoroughly by hand for approximately one minute. Hands, and the inside of the sink were then washed down and the surface of the water examined. Adults appearing on the surface were picked off and the soil stirred again for one minute.

The second and final examination lasted approximately two minutes, during which time the surface bubbles were systematically disturbed using a very thin piece of wood.

After five minutes treatment of the soil in this way no further adults were obtained even though the soil was on several occasions stirred at intervals over periods of many hours. Twenty adults which were put into soil were all recovered by this method.

It was observed that the majority of the adults obtained appeared on the surface of the water following the first thorough stirring. A small proportion of the adults appeared to have been crushed between particles of soil and these, dead or damaged, were found most frequently after the second stirring.

It is suggested that by the use of warm water, 30°C. approximately, more speedy results are obtained than when cold water is used. An observation that adults appear on the surface of warm water kicking vigorously whilst those on cold water mainly appear motionless, suggests that if warm, the mite is able to free itself from surface débris thus gaining the surface more readily.

The flotation method does not appear to be suitable for recovering large numbers of nymphs from the soil. The nymph, besides being more fragile than the adult, is also more easily wetted. Some do float to the surface, however, and these are most commonly found within the first two minutes.

Field Methods and Observations on Adults.

Each soil sample taken during this investigation near Compton was washed separately and represented soil taken to a known depth. From 14th July 1946 to the end of August periodic samples were taken, when possible, to a depth of eighteen inches and resulted in the recovery of 200 adults of *T. autumnalis*. All the positive results were from samples taken in the near vicinity of rabbit burrows on " chalk-down." Approximately half the samples were taken from soil under cover of short grass and small bushes ; the others were from points where the only cover was short grass.

Between 16th September and 12th December soil samples were taken within an area sixteen yards long by four yards wide as shown in fig. 4. The depth of soil above the solid chalk surface was almost uniform at twelve inches, therefore sampling was restricted to that depth. This small area to within a yard of the grass track was sheltered by the overhanging branches of trees constituting the hedge. The ground cover where samples were taken was short vegetation three to four inches high, fringed by bushes with the exception of the yard square plots on the extreme right of fig. 4 where the ground cover was thicker, taller and completely sheltered by small bushes.

Within each square shown in fig. 4, five samples of soil were taken to a depth of four inches, five samples from four inches to eight inches and five from eight to twelve inches. The total volume of soil taken in these fifteen samples was 2·25 cubic feet. The number of adults found in the area figured was 557.

Adults were recovered in large numbers only from soil samples taken within five yards of rabbit burrows ; of these, greater numbers were found beneath the cover of bushes than beneath short grass.

In fig. 4, it is noticeable that three yard squares on open ground are negative though only a short distance from the area where the distribution of adults is almost uniform.

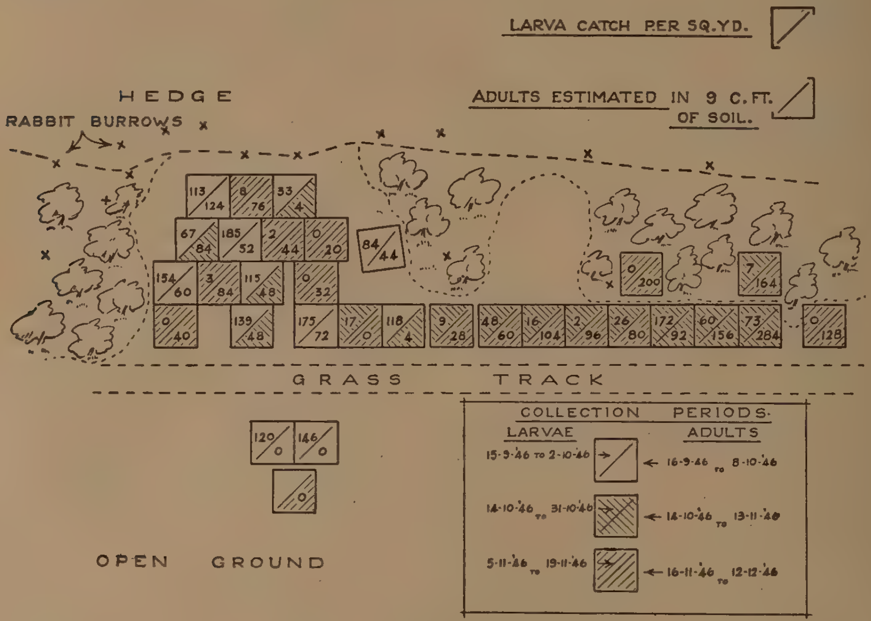

LARVA CATCH PER SQ.YD.
ADULTS ESTIMATED IN 9 C.FT. OF SOIL.
HEDGE
RABBIT BURROWS
113/124
8/76
33/4
67/84
185/52
2/44
0/20
84/44
154/60
3/84
115/48
0/32
0/200
7/164
0/40
139/48
175/72
17/0
118/4
9/28
48/60
16/104
2/96
26/80
172/92
60/156
73/284
0/128
GRASS TRACK
120/0
146/0
0
OPEN GROUND
COLLECTION PERIODS.
LARVAE
ADULTS
15-9-'46 TO 2-10-'46
16-9-'46 TO 8-10-'46
14-10-'46 TO 31-10-'46
14-10-'46 TO 13-11-'46
5-11-'46 TO 19-11-'46
16-11-'46 TO 12-12-'46

Fig. 4.

Observations on vertical distribution (Adults).

The following observations are based on a study of a chart showing the depth at which numbers of adults were found correlated with rainfall and maximum-minimum air temperature data.

(1) That the adults of *Trombicula autumnalis* rise towards the surface of moist soil when the air temperature is high, and go deeper into moist soil when air temperatures are near or at freezing point.

(2) The adult, under very dry conditions, appears to go deeper into the soil towards moisture and to rise after the first rain following a prolonged dry period.

(3) Adults near the surface of the soil under turf appear to go deeper after heavy or continuous rain, irrespective of air temperature.

On 14th July 1946, following a nine-day period without rain, when the twenty-four hourly maximum temperatures ranged from 64 to 83°F. and the minimum from 42 to 60°F., adults were only found between twelve and eighteen inches deep. The surface soil at this time was very dry and at a depth of eighteen inches only slightly moist. Following rainfalls of 5·2 millimetres on the 16th and 7·0 mm. on the 18th July a high proportion of adults found were between six and twelve inches deep, though the highest maximum temperature recorded between the 14th and 18th July was 65°F. and the minimum 44°F.

Throughout the following season the soil sampled was not so dry as on the 14th July and until the first frost, the vertical movements of adults appeared to correspond with the rise and fall of maximum temperatures.

Only on two occasions numbers of adults found between the surface and four inches deep were greater than those found at lower levels. These were 26th July and 4th August when the surface soil was moist and maximum temperatures reached 80°F.

Heavy and prolonged rainfall resulted in consistent negative samples from the 0–4-inch level even though temperatures were still high. The majority of adults were then found between 4 and 8 inches. Within 48 hours of the cessation of heavy rain when temperatures were high, adults were again recovered from 0–4-inch samples.

Following a minimum temperature of 29°F. on 25th October only an occasional adult was found in the upper levels until November when no further adults were found. Throughout November and until the 12th December when soil collections ceased, no adults were found in the first four inches, a few only between four and eight inches and large numbers between eight and twelve inches.

Field Observations on Larvae.

A suitable site for trapping experiments was found near Compton (see fig. 4) in a place which normally was overrun by rabbits but intensive trapping and shooting the previous year had reduced the population very considerably. Examination of the burrows in this area showed that only a small proportion remained inhabited. It was therefore probable that few larvae were picked up by their natural host.

It was not until 20th July that the first larva of *Trombicula autumnalis* was found after a widespread search of the chalk-down. This was caught in the field mite-trap which until the end of August was undergoing evolution. Consequently, no reliable indications as to the first appearance of larvae or seasonal increase in larval infestation were obtained. Very few were caught until near the end of August when numbers increased rapidly reaching a peak period about the 15th September. At this time it was possible to collect only a few larvae from boots or by laying white filter paper on the soil surface, and numbers up to 140 per square yard had been caught in the field mite-trap.

As shown in fig. 4, twelve plots, each of one square yard, were trapped during the peak period from 15th September to 2nd October. The ten plots trapped between 14th and 31st October show a very definite decline in infestation, whilst shortly after the 15th October catches became negative.

The fact that catches of 120 and 146 larvae are recorded from the two squares on open ground where no adults were found in the soil, suggests that larvae are inclined to wander in search of a host animal.

As there is no evidence of a decline in numbers of adults during the autumn season, and in view of the fact that numbers of animal hosts in this area had been so greatly diminished, it is reasonable to make a comparison between larvae caught and adults found in the soil.

The ratio between larvae and adults during the larval peak period is approximately two larvae to one adult. Over the period 14th to 31st October, the ratio is reversed to approximately 2·5 adults to one larva. It is realised that such figures give no true indication of the number of eggs laid by an adult during a season, but they do suggest that only a few eggs are laid at a time.

Notes on rearing *Trombicula deliensis.*

The following notes are extracted from an account of work done in the Scrub Typhus Research Laboratory, Imphal, between July and December 1945.

This work had two objects : (1) to produce nymphs which would be correlated with their larvae, for systematic purposes ; (2) to establish a technique for breeding larvae through adults to a second generation, so that mites in all stages of development might be available for experiments and for the establishment of hereditary transmission of infection.

Following the suggestion of Japanese workers that the *Trombicula* nymphs and adults were vegetarian, attempts were made to provide vegetarian and mineral food.

It was not until later that suspicions were aroused that the mites may be attacking each other, for the only nymphs to reach maturity were in tubes containing many mites. Isolated nymphs did not thrive.

It is now known from observations by several workers (Jayewickreme & Niles, 1946 ; Wharton & Carver, 1946 ; Richards, 1945 personal communication) that the Trombiculid nymphs and adults are predacious.

Routine rearing of nymphs for systematic purposes.

Fully engorged larvae in batches of 100 or so were placed in cloth-bottomed, shallow metal cells. After closing the cells with cover slips held in position by wax they were put into a petri dish humidor (as shown in fig. 5) and kept wet for the first twenty-four hours. The larvae appeared to become nymphophanes much more quickly if at first they were partially immobilised by a thin film of water. Using white cloth for the bottoms of the cells and completely covering the upper part of the humidor with black paper helped to keep the larvae from climbing to the cover slip where they would be caught in condensation.

After twenty-four hours the cells were transferred to a humidor in which the filter paper diaphragm was kept moist but not wet ; the black cover was retained on the upper part of the humidor. As soon as the larvae showed signs of undergoing metamorphosis, the cell was lightly flooded by placing it on a drop of water. This moistened the nymphophanes and floated them from the cloth complete with larval pelts. They were then picked off and placed singly in cells having bases made of black cloth in which, on emergence of the nymph, the larval pelts (exuviae) could be easily seen. Damage to the larval pelt was avoided by this flotation of the early nymphophane which prevented it from adhering to the substratum. Kept at a temperature of 30–32°C. the nymphs of *Trombicula deliensis* emerged in six and a half to eight days.

It was found that the nymphophanes and larval pelts were most easily handled by using a piece of soft wood shaved down to a feather edge.

After emergence, nymphs were then mounted (in polyvinyl alcohol mountant) for systematic study and correlation with the larval pelt, or, they were put singly or collectively into tubes of soil as described below.

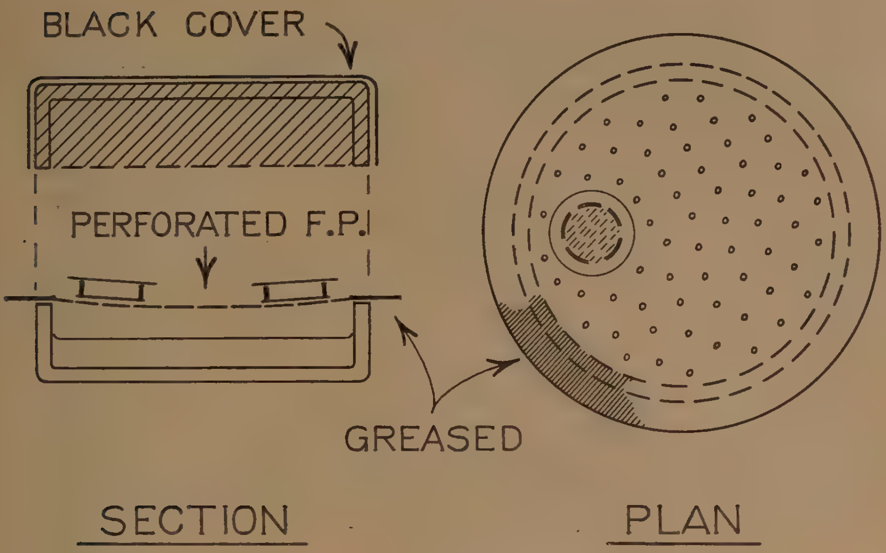


Fig. 5.—Petri dish humidor.

Observations on larvae and nymphs in tubes containing soil.

Batches of fully engorged larvae from the light-trap were put into tubes (3 in. × 1 in. and 2 in. × 1½ in.) which were quarter filled with very moist soil. The tubes were sealed with waxed corks, and black paper covers made to slip over the tops of the tubes, reaching a level ¼ inch below the soil surface. This kept the larvae from climbing up the tube and becoming trapped in the considerable amount of condensation which developed. After twenty-four or forty-eight hours, the tube was almost filled with dry soil and the black cover was replaced until the larvae had undergone metamorphosis. Nymphs of *T. deliensis* emerged in about seven days.

On the appearance of nymphs in the tube the black cover was reversed so that it blacked out the lower portion of the tube up to within a quarter of an inch of the soil surface. This succeeded in keeping the nymphs (which appear in the main to avoid bright light) within the soil. Those which did climb the tubes usually did so at night when the laboratory was dark.

Single nymphs in soil appeared noticeably larger after three weeks, but none of these matured to the adult stage. No adults appeared in tubes of soil containing cell-reared nymphs only. Adults were obtained in tubes of soil among a number of mixed mites. These were cell-reared nymphs, fully engorged and partially engorged larvae (*T. deliensis*).

Fungal mycelia occurred in many of the soils in tubes. The addition of lime gave only slight improvement, while the addition of humic acid appeared to encourage the growth. The least mycelial growth was found on a soil made up of 70 per cent. dark loam, 30 per cent. decayed leaves and grass, a trace of carbon, lime watered. Before use, the soil was sun-dried for several hours.

The proportion of mites which became adult was small and they appeared where no fungal mycelia were present. No adults bred from larvae, however, were found to have laid eggs.

A few larvae were obtained from eggs laid by an adult which had been recovered from the soil ; the larvae appeared after an interval of less than four weeks.

It was observed that nymphs usually became inactive in dry soil but were activated when a drop of moisture was put on the surface. Nymphs at the bottom of tubes were also activated although they were 2–2½ inches away from the moistened soil. Movements of nymphs were impeded on moist soil where adults moved with freedom.

Premature detachment of larvae from hosts was suspected where there had been rough handling. Most of the larvae from rats which were handled by grasping the skin of the nape of the neck with Cheatle's forceps and anaesthetised by bromethol injections, or from rats which fought each other in captivity or when placed together in the light traps, were distinctly smaller in size than the rotund larvae from rats which received little handling and only a cursory examination. It would appear that undue handling or other disturbance of partially engorged larvae may cause them to leave their host prematurely.

Laboratory observations on T. autumnalis *in England.*

I was able to confirm the observations of the aforementioned workers on the predacious habits of *Trombicula*. On two occasions nymphs were seen to feed on partially engorged larvae. These were in soil which was kept almost dry and it was at first thought that lack of moisture may have been the cause of their behaviour. Later, however, three other nymphs were observed to kill and feed on larvae in moist soil.

One nymph was seen to feed on another which was trapped in condensation, and on another occasion a nymph was observed feeding on a nymphophane.

Although larvae were put into tubes containing adults, no adults were seen to attack or feed on them.

Notes on technique.

Breeding cells were cut from ½ to ¾-inch aluminium tubing at eighth of an inch intervals. After smoothing the ends quite flat on a fine stone, a bevel was cut on the inner edge of the end chosen to be the top of the cell. After heating the ring, sealing wax was applied with a hot metal rod to the end destined to be the bottom of the cell, and the heated ring pressed on to cloth (*e.g.*, parachute silk) which was then trimmed to shape. Turning the cell upside down, the cloth was stretched taut by sliding, under pressure and in an outward radial direction, the heated end of an old scalpel. The top of the cell was waxed with low melting-point paraffin wax and a coverslip could then be readily sealed in place by running a hot rod over it. Mites were not harmed by sealing the cell thus, if the cloth bottom were kept moist. The bevel on the inner edge is important, as it prevents the wax from spreading over the inner surface of the coverslip when sealing it down.

It was found easier to place fully-engorged larvae in cells, or to transfer nymphophanes, if the cell bottom was lightly flooded. Nymphs should be picked up by a sweeping sideways approach, as otherwise they cling on to the cloth and can even be bent double without being dislodged.

Mounting larval pelts.—The exuviae are most easily seen and handled if the cloth bottom of the cell is quite dry. The softwood fine spatula should be moistened before use. Some of the best specimens of pelts were obtained by detaching them from the nymphs before the latter had time to brush them from their own backs.

The polyvinyl alcohol mountant used for the pelts contained picric acid. A pelt was placed on and saturated by the mountant. No care was taken at this stage to straighten the pelt and the coverslip was lowered on by withdrawing a needle from one side. The pelt was then located under the microscope and if too near the edge of the coverslip the opposite side was lightly boiled over a small flame. If the slide were then rapidly cooled by placing on a cold surface, the receding bubble drew the pelt from the edge towards the centre.

The pelt can be unrolled or turned over at will by this method, provided the bubble is controlled. Little damage to the pelt ensues, although the scutum sometimes floats clear of the rest of the pelt.

Acknowledgements.

The investigation, which was financed by the Medical Research Council, was begun when I was a member of the Friends Ambulance Unit. They allowed me to be seconded and proceed to South East Asia at the request of Dr. Kenneth Mellanby, O.B.E., who wished me to assist him in work on mite biology and who made it possible for me to undertake this research. I am indebted to Lt.-Col. J. R. Audy, R.A.M.C., for his encouragement when I was attached to his unit in Assam. Also to Major H. M. Thomas, R.A.M.C., for advice on technique. On returning to England I was appointed to assist Dr. W. S. Richards in his work on *T. autumnalis*, and he gave me helpful advice and opportunities for carrying out my own investigations. I am indebted to Dr. W. S. Gordon, Director of the A.R.C. Field Station, Compton, for the facilities which he provided at his Institute. Finally I wish to thank Professor P. A. Buxton, C.M.G., F.R.S., for his constant interest in this work and for all his help in producing the manuscript.

Summary.

The parasitic larvae of a number of Trombiculid mites are of economic importance as they include the vectors of scrub typhus, and scrub-itch mites of considerable nuisance value. Methods of trapping these mites and various field and laboratory observations on them are described. The methods were evolved and observations made during the course of work, sponsored by the Medical Research Council, in Imphal, Assam and Compton, Berkshire.

Live unfed larvae in the field may be concentrated and trapped by use of a low cover with a central light-admitting aperture and collecting apparatus. This method proved very efficient in trapping harvest mites (*Trombicula autumnalis*) in England. The rise in temperature within the trap can be made advantageous.

Guineapigs were chosen as bait animals for the collection of *T. autumnalis* at Compton, most of the larvae attaching near the anus and genitalia. The larvae fed for between 30 and 60 hours.

Engorged larvae may easily be collected as they leave their host (or dead animals and débris) by the funicular laboratory light-trap described.

Observations are made on the collection of adult mites, and to a lesser extent nymphs, from soil flotation in water. The vertical migration of adults in relation to soil moisture and temperature is discussed.

Observations on the appearance of larvae of *T. autumnalis* on a chalk-down at Compton showed a sharp seasonal peak in September, 1946, and some evidence is adduced that this was related to a peak in oviposition.

Observations are made on attempts to rear *T. deliensis*, the scrub typhus vector in Assam, and notes are added on the behaviour of larval and nymphal mites.

References.

JAYEWICKREME, S. H. & NILES, W. J. (1946). Successful feeding experiment with an adult Trombiculid mite.—Nature, **157,** p. 878.

Wharton, G. W. & Carver, R. K. (1946). Food of nymphs and adults of *Neoschöngastia indica* (Hirst 1915).—Science, **104,** pp. 76–77.

Williams, R. W. (1946). A contribution to our knowledge of the bionomics of the common North American chigger, *Eutrombicula alfreddugesi* (Oudemans) with a description of a rapid collecting method.—Amer. J. trop. Med., **26,** pp. 243–250.

ON THE OLD WORLD SPECIES OF THE GENUS *STETHORUS* WEISE (COLEOPTERA, COCCINELLIDAE).

By A. P. Kapur.
Commonwealth Institute of Entomology.

The genus *Stethorus* Weise is of special interest on account of its cosmopolitan distribution and the habit of its species of feeding mainly on mites, some of which are pests of crops, including fruit trees. Its relationship with other members of the tribe Scymnini is also of importance, as it was separated by Dobrzhansky in 1924 into a distinct tribe called Stethorini, which, however, was regarded by Korschefsky (1931) as synonymous with Scymnini. The material on which the present study is based came mainly from the Old World. Much of it had been submitted to the Commonwealth Institute of Entomology for identification, and I am grateful to Dr. W. J. Hall and Dr. T. H. C. Taylor, of this Institute, for giving me facilities and help. The remainder of the material studied was in the collection of the British Museum. I am indebted to Mr. N. D. Riley for procuring, on my behalf, certain type specimens from abroad for examination.

Geographical Distribution.

There are seven known species from the New World and twenty/ from the Old World. Of the latter, twelve are described as new in this paper, and eight other known species are described in addition. /-one

A. *New World Species.*

North America	*Stethorus atomus* Casey (Texas) *S. brevis* Casey (California) *S. picipes* Casey (California) *S. punctum* Lec. (Eastern North America, Puerto Rico) *S. utilis* Horn (Florida, Cuba)
South America	*S.* (*Nephopullus*) *darwini* (Brèthes) (Uruguay) *S. ogloblini* Nunenm. (Argentina)

B. *Old World Species.* (21)

Europe and Asia	*Stethorus gilvifrons* (Mulsant) *S. punctillum* Weise
Arabia	*S. pauperculus* Weise
Africa	*S. aethiops* Weise *S. jejunus* Casey *S. salutaris*, sp. n. *S. wollastoni*, sp. n.
Madagascar	*S. cruralis* Sicard *S. minutissimus* Sicard

Mauritius	*S. vinsoni*, sp. n.
India, Burma and Ceylon	*S. gilvifrons* (Mulsant)
	S. parcepunctatus, sp. n.
	S. pauperculus Weise
	S. rani, sp. n.
	S. rotundulus (Mots.)
	S. tetranychi, sp. n.
Malaya	*S. ~~malayensis~~*, sp. n.
	S. siphonulus, sp. n.
China	*S. aptus*, sp. n.
Philippine Islands	*S. pauperculus* Weise [?]
	S. rotundulus (Mots.) [?]
Australia	*S. nigripes*, sp. n.
	S. vagans (Blackburn)
New Zealand	*S. bifidus*, sp. n.
Fiji Islands	*S. fijiensis*, sp. n.

not mentioned

runcatus

12 sp. n.

The species are very small, usually between 1·0 mm. to 1·5 mm. in length. They are mostly black and devoid of any markings on the pronotum and elytra, so that, superficially, they resemble one another very closely. The structure of their male genitalia, however, offers reliable specific characters which it has been possible to correlate with certain external characters such as the punctation, pubescence, shape of the femoral lines and of the last abdominal sternite in the males. In many species the colour of the clypeus and of the legs seems to be fairly constant, but colour is not as useful a specific character as had been previously supposed. Several distinct species seem to possess similar coloration.

Feeding Habit and its Importance.

The following table shows the food of various species of *Stethorus* as recorded in the literature and as stated on the labels attached to the material under study. Certain species recorded in the literature (especially on economic entomology) as belonging to *Scymnus* have also been included because subsequent examination of the original material in such cases has proved that they belong, in fact, to *Stethorus*.

It will be observed that the majority of the species feed on mites, although in rare instances other kinds of food, such as Aphids and Thrips, have also been reported. Critical examination and feeding experiments in such cases may reveal, however, that mites are the principal food of all species.

There are several instances in which *Stethorus* species are reported to have effectively reduced the population of mites occurring as pests. Uspenskiĭ (1937) recorded that *S. punctillum* Ws. considerably reduced the infestation of *Tetranychus turkestani* Ugar. and Nik. on cotton in Uzbekistan, and that in 1935 it completely eliminated this mite on cotton in Samarkand. The same species of *Stethorus* was reported to have rapidly reduced the population of *Tetranychus telarius* L. in Tashkent (Radzievskaya, 1931) and also to have exercised, from time to time, some control of it in vineyards in Europe. In India, *S. pauperculus* Ws. and *S. gilvifrons* (Muls.) (which have sometimes been erroneously recorded as *Scymnus gracilis* Mots.) exercise a valuable check on the population of mites on various crops such as sorghum (*cholam*), castor, beans, apples, etc. In New Zealand, *Stethorus bifidus*, sp. n. (a fuller account of the biology of which was published by Cottier, 1934, who referred to it as *Scymnus* sp., near *minutulus* Broun) is an important predator of *Paratetranychus pilosus* C. & F. In North America, *S. punctum* Lec. was reported to have materially reduced the population of *P. pilosus* on fruit trees (Gilliatt, 1935; Hauser & Cutright, 1941).

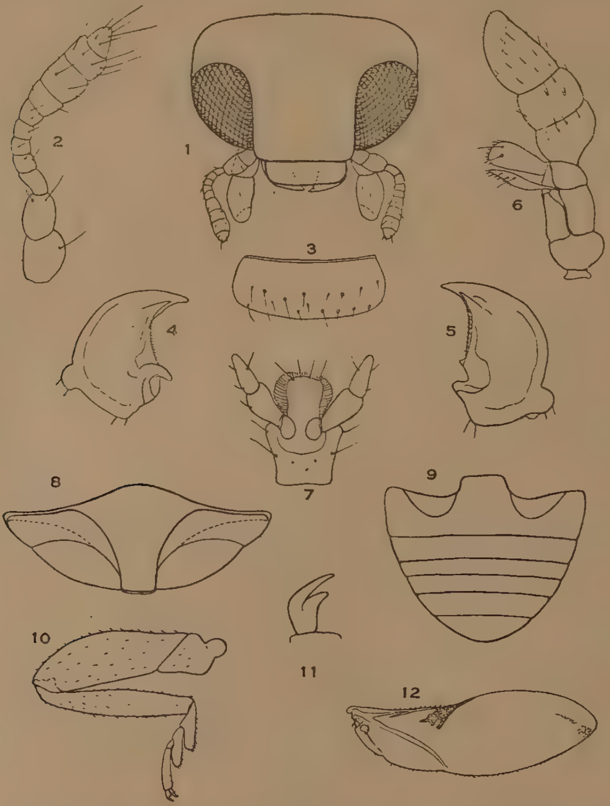
Stethorus species	Host	Plant	Country	Reference
S. punctillum Ws.	Mites *Tetranychus telarius* L.	Hops ...	Bohemia ...	Blattný & Balaček, 1924
,, ,,	,, ,,	Cotton ? ...	Tashkent, U.S.S.R.	Radzievskaya, 1931
,, ,,	,, ,,	Sugar beet ...	Italy ...	Menozzi, 1934
,, ,,	,, ,,	Grape vines	Holland and Belgium (glasshouse)	Vanwijngaerden, 1934; van der Helm, 1935
,, ,,	,, ,,	Hops, hydrangeas and roses	Britain (glasshouse)	Jary, 1935 ; Speyer, 1935
,, ,,	,, ,,	Lemon ...	Sicily ...	Martelli, 1937
,, ,,	*T. telarius* var. *russeolus* Koch	Castor ...	Italy ...	De Stefani, 1919
,, ,,	*T. turkestani* Ugar. & Nik.	Cotton ...	Uzbekistan, U.S.S.R.	Uspenskiĭ, 1937
,, ,,	*Paratetranychus pilosus* C. & F.	Fruit trees ...	Finland ...	Listo, Listo & Kanervo, 1939
,, ,,	Aphid : *Phorodon humuli* Schr.	Hops ...	Britain ...	Massee, 1940
,, ,,	Thrips	*Sambucus nigra*	Europe ...	Schaufuss, 1916
S. punctum Lec.	Mites : *Tetranychus telarius* (*bimaculatus*, Harvey)	Cotton ...	U.S.A. ...	McGregor, 1913 ; McGregor & McDonough, 1917
,, ,,	*Paratetranychus pilosus*	Fruit trees ...	U.S.A. ...	Garman, 1923 ; Ross & Robinson, 1922 ; Hamilton, 1924
,, ,,	,, ,,	,, ...	U.S.A. ...	Hauser & Cutright, 1941
,, ,,	,, ,,	—	Canada ...	Gilliatt, 1935
S. picipes Casey	*T. telarius*	Cotton ...	U.S.A. ...	McGregor & McDonough, 1917
,, ,,	*Tetranychus* sp. ...	Citrus ...	U.S.A. ...	Quayle, 1913
,, ,,	*P. pilosus*	Citrus ...	U.S.A. ...	Borden, 1922
S. utilis Horn ...	*T. telarius* (*bimaculatus*)	Cotton ...	U.S.A. ...	McGregor & McDonough, 1917
,, ,,	*T. yothersi* McG. ...	Avocado ...	U.S.A. ...	Moznette, 1919 and 1922
Stethorus sp. ...	*T. pacificus* McG. ...	Apple ...	U.S.A. ...	Webster, 1942
S. vagans (Blackb.)	*Tetranychus exsiccator* Zhnt.	Sugar-cane ...	Hawaii ...	Swezey, 1923
,, ,,	*Bryobia praetiosa* Koch	Fruit trees, clover and grasses	Tasmania ...	Evans, 1943
S. bifidus, sp. n.	*Paratetranychus pilosus*	Apple trees	New Zealand	Cottier, 1934 (see *Scymnus* sp.)
S. salutaris, sp. n.	Eggs of mites ...	—	West Africa	Hargreaves, 1932 (see *Stethorus jejunus* Casey)
S. *pauperculus* Ws.	*Paratetranychus indicus* Hirst	Sorghum ...	South India	Cherian, 1933 (see *Scymnus gracilis* Mots.)
,, ,,	Mites	Castor, *Andropogon* and plantain	India ...	In collection
S. gilvifrons (Muls.)	Mites	Apple tree and castor	North India	,,
S. tetranychi, sp. n.	*Tetranychus* sp. ...	Jute ...	Bengal, India	,,

Description of the Genus.

Stethorus Weise, 1885 (Type : *Stethorus punctillum* Weise).

Nephopullus Brèthes, 1924 (Type : *Nephopullus darwini* Brèthes). **(syn. nov.)**

Referable to the tribe Scymnini. Small, usually 1·0–1·5 mm. long, oblong-oval to rounded, moderately to strongly convex. Black, except the antennae and mouthparts and, in certain species, the clypeus and legs also, all of which vary from piceous-red to yellowish-testaceous. Head (fig. 1) transverse, subquadrangular, with large, moderately coarsely faceted eyes ; epicranium usually covered by the pronotum ; clypeus subparallel laterally, slightly produced forward beyond the anterior margin of the eyes, truncate, anteriorly with the antero-lateral angles sub-rounded but not emarginate round the base of the antennae ; antennae (fig. 2) rather short, eleven-segmented, clubbed, with the first two segments large and oblong, the third narrower but longer than the next three or four which are nearly as wide as the third, the terminal four wider, together forming the club ; labrum (fig. 3) transverse, with the



Figs. 1–12.—*Stethorus punctillum* Weise. (1) front of head ×75 ; (2) antenna ×162 ; (3) labrum ×162 ; (4 & 5) mandibles ×162 ; (6) maxilla ×162 ; (7) labium ×162 ; (8) prosternum ×75 ; (9) abdominal sternites of the female ×34 ; (10) leg ×75 ; (11) claw ×162 ; (12) wing ×18.

lateral margins sub-rounded ; mandibles (figs. 4 and 5) bifid at the apex, with the inner tooth much smaller than the outer one, the basal tooth bicuspidate and with a grinding surface near its base over which the basal tooth of the opposite mandible operates ; maxilla (fig. 6) moderately developed except for the spatulate galea ; the maxillary palpus with the terminal segment oblong, obliquely truncate and narrower towards the apex ; labium (fig. 7) small with rather long palpi ; the second and third segments of the palpus club-shaped and conical, respectively. Pronotum transverse, nearly twice as broad as long in the middle, with the anterior margin moderately to deeply emarginate, arcuate laterally and much shorter than the basal margin ; the lateral margins moderately rounded, forming acute angles with the anterior margin ; the base weakly emarginate on either side of the truncate median part opposite the scutellum. Both the lateral and the basal margins are narrowly margined. Scutellum usually small and triangular, always with a few fine punctures and short hairs. Elytra with the base nearly as wide as that of pronotum, with the shoulder angles rather acute, the callus slightly prominent, and the elytral epipleurae moderately wide and devoid of any foveae. Membranous wings as shown in figure 12. Prosternum (fig. 8) without any carinae ; convex medially and produced forward in the middle in the form of a broad arch, which only partly covers the mouth-parts. There are six visible abdominal sternites (fig. 9), the first nearly twice as long as any one of the subsequent four and with usually complete and weakly to strongly arched femoral lines ; the last sternite entire in the female but in the male varying from entire to strongly emarginate or truncate posteriorly. Femora (fig. 10) moderately developed, not expanded but slightly channelled to accommodate a part of the base of the slender and compressed tibiae ; claws bifid (fig. 11).

Sclerotised parts of male genitalia similar to those of other Coccinellids ; sipho varying from a short, stout, slightly curved tube (fig. 71) to a long, thin, whip-like (fig. 51) structure, its proximal end, called the siphonal capsule (fig. 14, sc.), differing in shape, in closely related species ; basal plates (fig. 13, bp.) usually fused in the form of a ring through which the sipho passes ; penis (fig. 13, p.), which is in fact a posterior extension of the basal plates, consisting of a rolled piece which may be conical or tubular and varying considerably in shape in different species or groups of species ; paramera (fig. 13, pa.) arising as paired structures from the basal plates, and trab (fig. 13, t.) varying considerably in length but usually thickened at apex. In the female, the receptaculum seminis is not usually sclerotised, and the ninth sternite, which is divided so that it is actually a paired structure, forms a short tube and varies in shape in different groups of species.

Regarding *Nephopullus*, Brèthes (1924), it may be remarked that the genotype, *N. darwini* Brèthes, from Uruguay, as fixed by Korschefsky (1931), which is in the British Museum, belongs to *Stethorus* as it possesses all the important characters of the genus. Recently Nunenmacher (1937) described *Stethorus ogloblini* Nunenm. from Argentina, which shows that *Stethorus* occurs in South America. There appears to be little justification for erecting a separate genus for the South American species.

Taxonomic Position of the Genus.

Stethorus was originally proposed by Weise (1885) as a subgenus of *Scymnus*, but in 1899 both Weise and Casey regarded it as a valid genus. However, in some later works, it has been and is still being regarded as a sub-genus of *Scymnus*. On the other hand, Dobrzhansky (1924, 1926) did not consider it as a member of the tribe SCYMNINI and proposed for it a distinct tribal name, STETHORINI ; this was mainly on account of the peculiar structure of the female genitalia of the (genotype) species, *S. punctillum* Ws., which has only two ovarioles and is devoid of bursa copulatrix, receptaculum seminis and accessory glands. The male genitalia were also reported by him to be different from the form usually found in the family but no details of the differences were given. Korschefsky (1931), in his catalogue, synonymised STETHORINI with

Scymnini. With increased knowledge of the structure of the family as a whole, and of the large number of species of the genus, the above-mentioned character of the female genitalia does not appear to be quite so peculiar nor so universal within the genus as to warrant its separation into a distinct tribe. The male genitalia, in my opinion, do not show any peculiarity, and all gradations in the shape of the sipho, from a coiled, whip-like structure to a short and stout tube, are met with in the various species. In the female, the absence of the receptaculum seminis, etc., though very common, is by no means universal. In *Stethorus bifidus*, sp. n., from New Zealand, a well-developed and strongly sclerotised receptaculum seminis is present. Besides, this structure is known to be absent in several other Coccinellids which are not related to one another, and is, therefore, of little significance in so far as the erection of separate tribes is concerned. However, it must be emphasised that there are several other external characters in *Stethorus* which make it difficult to establish its relationship with the other genera of the tribe Scymnini, all of which have an exposed antennal base round which the clypeus is emarginate. In *Stethorus* the antennae arise immediately between the eyes and the lateral margins of the clypeus, and although the antennal base is exposed, the clypeus is not emarginate round the base. The other important difference lies in the shape of the prosternum which, in *Stethorus* and in no other genus of the tribe, is convex and produced forward in an arc in the middle. In *Bucolus* Muls. the middle part of the prosternum is produced forwards but it is doubtful whether this genus belongs to Scymnini at all, as it has only five abdominal segments, and has the elytral epipleurae deeply foveolate. As regards *Cryptolaemus* Muls., its whole prosternum is greatly enlarged and produced forward to cover the mouth-parts completely; moreover, it has a pair of short prosternal carinae. The characters of *Stethorus* that are also found in other genera of the tribe Scymnini are: the pubescent body, the less coarsely faceted eyes, the short and eleven-segmented antennae, the narrow clypeus and six visible abdominal sternites. On on account of the biological and structural peculiarities already mentioned, *Stethorus* may be regarded as an aberrant genus of the tribe Scymnini.

Description of the Species.

Stethorus punctillum Weise (figs. 1–17).

Stethorus punctillum Weise, 1891, p. 781; Casey, 1899, p. 136; Mader, 1924, p. 15; Korschefsky, 1931, p. 112.

Stethorus punctillum var. *investitus* Roubal, 1920, p. 79.

Stethorus investitus (Roubal), Mader, 1924, p. 15; 1927, p. 193.

Coccinella minima Rossi, 1794, p. 89; (preoccupied, *Coccinella minima* Müller, 1776, p. 65); Harold, 1875, p. 118.

Stethorus minimus (Rossi), Weise, 1885, p. 74; 1891, p. 781.

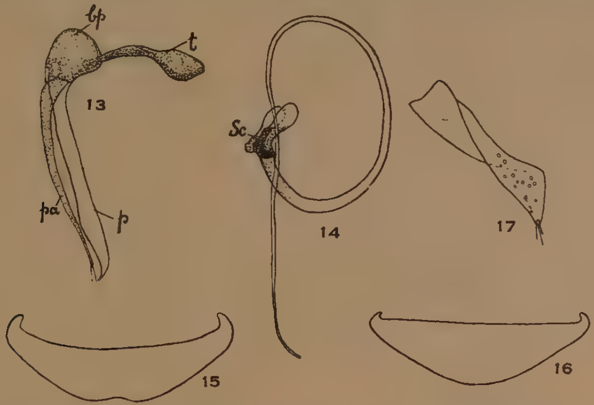
Coccinella pusilla Herbst, 1797, p. 346; (preoccupied, *Coccinella pusilla* Müller, 1776, p. 65).

Scymnus ? *pusillus* (Herbst), Crotch, 1874, p. 308; Harold, 1875, p. 118.

Coccinella atra Illiger, 1798, p. 413; (preoccupied, *Coccinella atra* Gmelin, 1789, p. 1664); Gyllenhal, 1827, p. 195.

Stethorus ater (Illiger), Korschefsky, 1931, p. 113.

Shortly oval, widest in the middle, moderately convex. Black except the testaceous antennae, mouth-parts, apices of femora, tibiae and tarsi. Dorsal surface with greyish, moderately long, sparse and semi-erect pubescence. Head with fine and sparse punctation; antennae nearly as long as the width of the clypeus and with a fusiform club (fig. 2); pronotum unevenly punctate, the punctures being fine and sparse in the median part and coarse, subrounded, navel-like and close together towards the lateral margins ; elytral punctures fairly coarse, impressed and close. Underside with greyish, short, rather sparse and subdepressed pubescence and with coarse and moderately close punctation except on the prosternum, elytral epipleurae and legs. Femoral lines (fig. 9) in the shape of a wide arch which extends as far as the middle of the first abdominal sternite ; the second sternite shorter than the latter and nearly as long as the fifth but slightly longer than the third or fourth which are subequal ; the sixth sternite in the male widely and distinctly emarginate in the middle of the posterior margin. Male genitalia : sipho (fig. 14) with a distinct, strongly sclerotised siphonal capsule which is bifurcate distally ; remaining part of sipho long, slender and becoming gradually narrower towards the distal end and forming a broad loop at the proximal end. Penis in the form of an elongate tubular structure with a sharply pointed apex which, in side-view, appears to bend towards the paramera ; paramera very narrow, a little shorter than penis, and provided with a couple of short setae at the apex ; trab short, nearly half as long as penis and expanded distally. Proximal part of siphonal capsule, paramera, basal plates and trab dark-coloured. Each half of ninth sternite in female subtriangular and bearing a number of short setae at the apex.



Figs. 13–17.—*Stethorus punctillum* Weise. (13) parts of the male genitalia : t, trab ; bp. basal plates ; p, penis ; pa, paramera ×75 ; (14) sipho, sc, siphonal capsule ×75 ; (15) sixth abdominal sternite of the male ×75 ; (16) the same of the female ×75 ; (17) ninth sternite of female ×75.

Length 1·3–1·5 mm., width 1·0–1·1 mm.

Geographical Distribution :—The distribution given by Korschefsky (1931) is Europe, Asia, Siberia and Japan. The examples examined are from various European countries, including Greece and Hyères island in the Mediterranean.

Stethorus gilvifrons (Mulsant) (figs. 18–22).

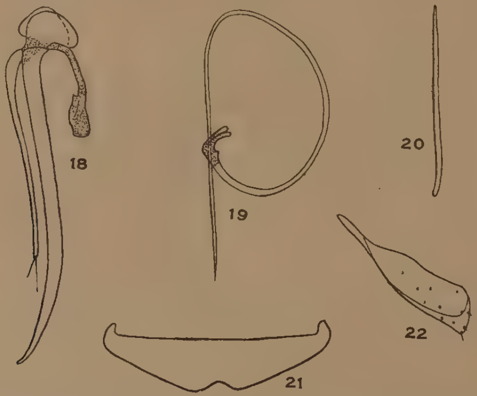
Scymnus (*Pullus*) *gilvifrons* Mulsant, 1850, p. 995.

Stethorus gilvifrons (Mulsant) Weise, 1885, p. 74.

Shortly oval, widest in the middle and slightly narrowed at the apex, convex. Head black except the testaceous anterior half to two-thirds of the interocular space, clypeus, antennae and mouth-parts ; pronotum, elytra and the underside black, legs testaceous, sometimes the femora of the last pair of legs castaneous or lightly piceous in the middle. Dorsal surface with greyish, moderately long, sparse and semi-erect pubescence. Head finely and sparsely punctate, antennae with fusiform club.

Pronotum unevenly punctate, the punctures fine and rather sparse in the median one-third and much coarser, navel-like and close together towards the lateral margins. Elytra with fine and fairly close punctures which are slightly more impressed in the apical half and are oblique (as if impressed from behind). Underside with greyish, short, sparse and subdepressed pubescence. Metasternum flat in the middle, finely and sparsely punctate, towards the lateral margins with coarse and close punctures; remaining parts of underside with rather fine and moderately close punctures. Femoral lines evenly rounded, wide and not extending beyond two-fifths of the length of the segment. The sixth abdominal sternite (fig. 21) in the male with distinct and wide emargination in the middle of the posterior margin. Male genitalia: sipho (fig. 19) very thin, long and forming a complete loop proximally; siphonal capsule dark, without any outer branch, but with the inner branch elongate, rather narrow and bifurcate at apex; penis (fig. 18) very long, nearly three times the length of trab, narrow and tapering towards the apex and bent towards the paramera in the apical one-third; the spicule (fig. 20) long and thin; paramera very narrow and nearly two-thirds the length of penis, each bearing a pair of short setae at the apex; trab short and expanded distally. Each half of ninth sternite (fig. 22) in female spatulate and provided with three or four short, widely separated setae towards the apex.

Length 1·25–1·4 mm., width 0·9–1·0 mm.



Figs. 18–22.—*Stethorus gilvifrons* (Muls.): (18) parts of male genitalia ×75; (19) sipho ×75; (20) the spicule ×75; (21) sixth abdominal sternite of the male ×75; (22) ninth sternite of the female ×75.

Geographical Distribution and Remarks:—The type locality is Derbent, Caucasus. Korschefsky gives the distribution as Asia Minor and South Europe. He also synonymises *S. minimus* Wollaston (not Rossi), from Madeira and the Canary Islands but examination of the material in Wollaston's collection in the British Museum shows that it is quite distinct from *gilvifrons*, and also from *punctillum* (syn. *minimus* Rossi). It is described below as a new species under the name *S. wollastoni*. The similarity in coloration, especially of the testaceous anterior part of the head, between the latter species and *gilvifrons* is misleading. The material examined was as follows:—

Mediterranean Region: Sardinia, Cagliari; Cyprus, Limassol, –.ii.1934 (*Mavromoustakis*); Hedeva (*sic*), 4.viii.1932; on red spider.

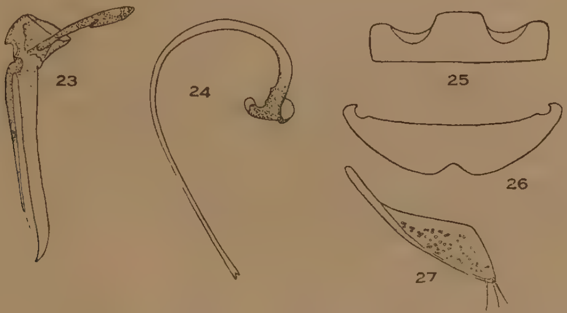
India: Baluchistan, Mastung, 27.vii.1938, predacious on mite on apple tree (*A. P. Kapur*); Punjab, Lyallpur, 23.iii.1935, predacious on mites (*M. Afzal Husain*); Lyallpur, 1.viii.1935, predacious on mites on castor leaves (*A. P. Kapur*).

Stethorus wollastoni, sp. n. (figs. 23–27).

Stethorus minimus Wollaston (not Rossi), 1854, p. 470.

Shortly oval, distinctly narrower than *Stethorus gilvifrons* (Muls.), moderately convex. Coloration as in *gilvifrons*, but the last pair of femora invariably deep brown to piceous. The pubescence on the dorsal surface greyish, fairly long and sparse. Head with rather coarse, impressed and sparse punctures; antennae with fusiform club. Pronotum unevenly punctate, the median one-third with fine and sparse punctures and the lateral parts with coarse, navel-like and moderately close punctures. Elytral punctures moderately coarse, less impressed and sparse. Surface between punctures smooth and shining. Underside with greyish, short and sparse pubescence, and with moderately coarse and sparse punctures except close to the lateral margins of the metasternum. Femoral lines (fig. 25) widely rounded, and extending as far as two-fifths of the length of the first abdominal sternite. In the male, the last abdominal sternite has a distinct emargination in the middle of the posterior margin (fig. 26). Male genitalia: sipho (fig. 24) moderately long, narrow in a regular arch proximally; siphonal capsule mostly dark, with the outer branch short and wide and the inner one longer and narrower; penis (fig. 23) tubular, nearly twice as long as the trab, gradually but slightly narrowing distally with the extreme apex slightly bent towards the paramera, which are slender, about three-fourths the length of penis and each provided with a short seta at the apex. The trab, basal plates and the proximal halves of penis and paramera dark. Each half of ninth sternite (fig. 27) in female subtriangular, with numerous irregularly crowded punctures and with three moderately long setae at the apex.

Length 1·3 mm., width 0·9 mm.



Figs. 23–27.—*Stethorus wollastoni*, sp. n.: (23) parts of male genitalia ×75; (24) sipho ×75; (25) first abdominal sternite ×34; (26) sixth abdominal sternite of the male ×75; (27) ninth sternite of female ×75.

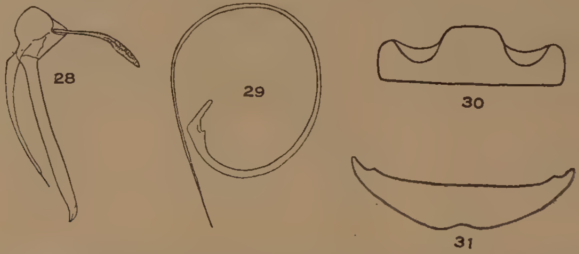
TYPE. One male in the Wollaston collection (British Museum) from MADEIRA.

PARATYPES. Both sexes, in the British Museum; 12 from Madeira and 6 from the Canary Islands, mostly in Wollaston collection.

REMARKS. The species is easily distinguished from *S. punctillum* Ws. by the testaceous coloration of the anterior part of the head, legs, etc., and by the less coarse and distinctly sparse punctation of the elytra. Further, the femoral lines also do not reach the middle of the segment as in *punctillum*. From *gilvifrons*, with which it agrees in the coloration of the head, etc., and in the shape of the femoral lines, it differs in being slightly narrower, in the punctation of the head and elytra, and in the structure of the genitalia.

Stethorus salutaris, sp. n. (figs. 28–31).

Shortly oval, fairly wide in the middle, strongly convex. Black, except the reddish-testaceous anterior part of the clypeus, the femora and the last tarsus of each leg and the testaceous antennae, tibiae and the remaining tarsi. Dorsal surface with greyish, long, moderately stout, semi-erect and sparse pubescence. Head finely and sparsely punctate ; pronotum unevenly punctate, punctures on the whole moderately coarse and navel-like but sparse in the middle and closer together towards the lateral margins ; elytral punctures coarse, fairly impressed and close ; surface between the punctures smooth and shining. Underside with greyish, rather short, sparse and subdepressed pubescence ; meso- and metasternum as well as the abdominal sternites coarsely and fairly closely punctate and the rest of the underside finely and sparsely punctate. Femoral lines (fig. 30) wide, evenly arched, and extending to just near the middle of the length of the segment. The sixth abdominal sternite (fig. 31) in the male with a wide and distinct emargination at the apex. Male genitalia : sipho (fig. 29) long, slender and curved proximally to form a loop ; siphonal capsule bulbous near the base and with an elongate inner branch ; the trab black, narrow, nearly half the length of penis and only moderately expanded distally ; penis (fig. 28) like an elongate tube which is sharply pointed at the extreme apex ; paramera nearly two-thirds the length of penis, moderately stout near the base and gradually narrowing towards the apex which bears a short seta.



Figs. 28–31.—*Stethorus salutaris*, sp. n. : (28) parts of the male genitalia ×75 ; (29) sipho ×75 ; (30) first abdominal sternite ×34 ; (31) sixth abdominal sternite of male ×75.

Length 1·4 mm., width 1·05 mm.

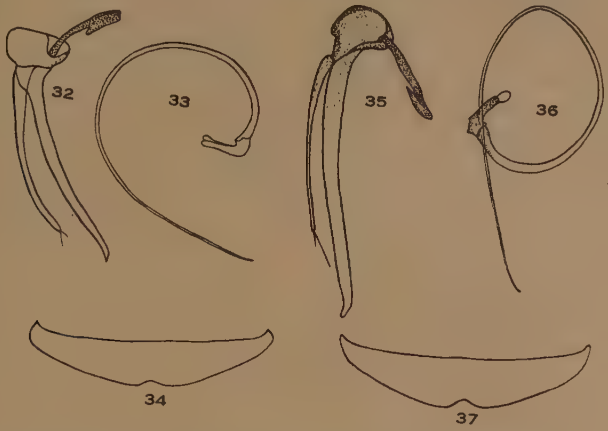
Type and one paratype. In the British Museum ; West Africa : Sierra Leone, predacious on eggs of mites, 21.viii.1926 (*E. Hargreaves*) (♂ genitalia of the paratype dissected).

Remarks. Hargreaves (1932) records this species as feeding upon eggs of mites under the name *Stethorus jejunus* Casey, which is quite different in structure and much smaller in size. *S. wollastoni* is very closely related to it but differs in the pubescence, punctation, and the structure of the male genitalia.

Stethorus aethiops Weise (figs. 32–34).

Stethorus aethiops Weise, 1899, p. 64.

Shortly oval, widest in the middle, moderately convex. Black, except the anterior part of the interocular space, clypeus, antennae, mouth-parts and legs, all of which are usually reddish-testaceous. Femora sometimes rather piceous except at the apices ; tibiae and tarsi pale testaceous. Dorsal surface with greyish, long, semi-erect and sparse pubescence. Head very finely and sparsely punctate ; pronotum unevenly punctate, the median part with fine and sparse and the lateral parts with coarse, navel-like and close punctures ; elytral punctures fine, impressed and sparse ; surface between punctures fairly smooth and shining. Underside with greyish, short and sparse pubescence ; punctation on the whole moderately coarse and close except on the prosternum and the median flat part of the metasternum where it is sparser and a little finer. Femoral lines comparatively less wide than in *gilvifrons*, semicircular and not quite reaching the middle of the length of the segment ; sixth abdominal sternite (fig. 34) in the male rather weakly emarginate at apex. Male genitalia : sipho (fig. 33) moderately long and narrow, subrounded at the proximal and straight at the distal end ; siphonal capsule without the external branch, the inner branch elongate and bifurcate ; the trab (fig. 32) piceous, short, stout and expanded at the distal end ; penis tubular, gradually narrowing distally and pointed at the extreme apex : paramera about three-fourths as long as penis, very narrow in the apical two-thirds and with a pair of short, delicate setae at the apex.



Figs. 32–34.—*Stethorus aethiops* Weise : (32) parts of male genitalia ×75 ; (33) sipho ×75 ; (34) sixth abdominal sternite of male ×75.
Figs. 35–37.—*Stethorus vinsoni*, sp. n. : (35) parts of male genitalia ×75 ; (36) sipho ×75 ; (37) sixth abdominal sternite of male ×75.

Length 1·5 mm., width 1·06 mm.

Geographical Distribution :—The type locality is Kwai, Tanganyika. Examples examined from the following localities :—

Tanganyika : Mafia Island, predacious on red spider on aster, –.vii.1936 (*D. Vesey Fitzgerald*).

Cape Province : Simonstown, 12–20.iv.1915 (*M. Cameron*) ; Constantia, –.iv. 1926 (*R. W. E. Tucker*).

Remarks. Closely resembling *S. salutaris* in shape, size, coloration, etc., but differs considerably in the punctation and the shape of the male genitalia, etc. Sicard (1909, p. 148) suspected it to be the same as *S. jejunus* Casey, which has apparently similar distribution but is much smaller in size and also differs in coloration, etc.

Stethorus jejunus Casey.

Stethorus jejunus Casey, 1899, p. 136.

Shortly oval, moderately convex. Black except the testaceous antennae, mouth-parts, apices of femora, tibiae and tarsi and the piceous-red remaining parts of the leg. Dorsal surface with greyish, moderately long, sparse and semi-erect pubescence. Head with very fine and sparse punctures ; pronotum unevenly punctate, the punctures being fine, irregularly dispersed and sparse in the median one-third and coarse, navel-like and close towards the lateral margins ; elytral punctures moderately coarse, impressed and rather close ; surface between punctures being smooth and shining. Underside with greyish, short and sparse pubescence ; pro- and meso-sternum with fine and sparse punctures ; metasternum with coarse punctures which are rather sparse in the median part and very close towards the lateral margins ; abdominal sternites coarsely and closely punctate. Femoral lines semicircular in outline and extending as far as two-thirds the length of the first abdominal sternite. Last abdominal sternite in male not emarginate but entire. Male genitalia : sipho short and stout, pointed at the apex and slightly bent near the proximal end ; siphonal capsule with only a subtruncate inner branch ; penis triangular, moderately wide at the base and nearly twice as long, gradually narrowing towards the apex which is more or less pointed ; paramera about two-thirds the length of penis, rather spatulate and provided with a few moderately long setae towards the apex.

Length 1·15 mm., width 0·8 mm.

Geographical Distribution :—The type locality is Cape of Good Hope (Cape Town). Examples examined from Tanganyika, Mafia Island, 3.vii.1936 (*D. Vesey Fitzgerald*).

Remarks. I have not been able to examine the type of this species, but as far as can be judged from the description, the material from Tanganyika belongs to it. In the structure of the male genitalia it resembles the Indian species, *S. pauperculus* Ws., which also occurs in Arabia. From *S. aethiops* Ws., which has a similar distribution, it is easily distinguished by its smaller size and by differences in coloration, punctation and the structure of the male genitalia.

Stethorus minutissimus Sicard.

Stethorus minutissimus Sicard, 1909, pp. 146, 148.

Shortly oval, fairly wide in the middle, strongly convex. Black, except the reddish-testaceous anterior part of the clypeus, the testaceous antennae, mouth-parts, apices of femora, tibiae and tarsi and the piceous remaining part of the femora. Dorsal surface with greyish, moderately short, dense and semi-erect pubescence. Head minutely and sparsely punctate ; pronotum with rather coarse and navel-like punctures which are finer and sparser in the middle than towards the lateral margins ; elytral punctures rather coarse but less so than those of the pronotum, shallow and irregular in outline, and only moderately close ; surface between punctures smooth and shining. Underside with the pubescence greyish, short, sparse and subdepressed. The prosternum, elytral epipleurae and last four abdominal sternites with fine punctures. On the remaining parts, the punctures rather coarse and sparse. The flat median part of the metasternum has irregular transverse striae. The femoral lines broadly rounded and not extending beyond two-fifths of the segment. Last abdominal sternite in male with a small but distinct emargination at the apex.

Length 1·25 mm., width 1·0 mm.

Geographical Distribution :—Madagascar (Type locality). Type, a male, in the Muséum Nationale d'Histoire naturelle, Paris.

Remarks. In many important external characters this species resembles *S. vinsoni*, sp. n., from Mauritius, but differs clearly from the latter by the coarser and sparser punctation of the elytra and also in the character of the punctation on the metasternum. The genitalia of the male type, which is unique, could not be dissected for obvious reasons.

Stethorus cruralis Sicard.

Stethorus cruralis Sicard, 1909, pp. 147, 148.

Examples of this species have not been seen and enquiries made regarding its type revealed that this is not present in the Muséum National d'Histoire naturelle, Paris. From the original description it follows that it would be the largest species of the genus, being 1·75 mm. long. The metasternum is also stated to be very convex, which is also quite unusual for the genus.

Stethorus vinsoni, sp. n. (figs. 35–37).

Shortly oval, fairly wide in the middle, convex. Black, excepting the testaceous clypeus, antennae, mouth-parts, apices of femora, tibiae and tarsi and the piceous to reddish-testaceous remaining parts of the legs. Dorsal surface with greyish, short and dense pubescence. Head minutely and sparsely punctate; pronotum with coarse, navel-like punctures which are only a little sparser in the middle than towards the lateral margins; elytral punctures, fine, impressed and close; surface between punctures more or less smooth and partly shining. Underside with the pubescence similar to that on the dorsal side, the punctation uniformly coarse and close except on the elytral epipleurae, the last three abdominal sternites and the legs, where the punctures are finer and sparser. Femoral lines broadly rounded, and not extending beyond two-fifths of length of segment; the last abdominal sternite in the male with a weak emargination at the apex (fig. 37). Male genitalia: sipho (fig. 36) long and thin and forming a loop proximally; siphonal capsule dark, except at the apex of the elongate inner branch, the outer branch absent; penis (fig. 35) long, tubular, slightly narrowing towards the apex, near which it is obtusely bent towards the paramera; trab short and stout, expanded at the distal end, nearly one-third the length of the penis; paramera very narrow, nearly two-thirds the length of the penis and each with a pair of short setae at the apex; the trab, basal plates, proximal one-fourth of penis and paramera dark.

Length 1·3 mm., width 1·05 mm.

TYPE. In the British Museum; MAURITIUS, Palmar, –.viii.1934 (*J. Vinson*).

PARATYPES. In the British Museum, in Mr. Vinson's and in my collection; Mauritius, Candros Is., 1946 (*David Madge*).

REMARKS. This species is closely related to *S. minutissimus* Sic. with which it has been compared above.

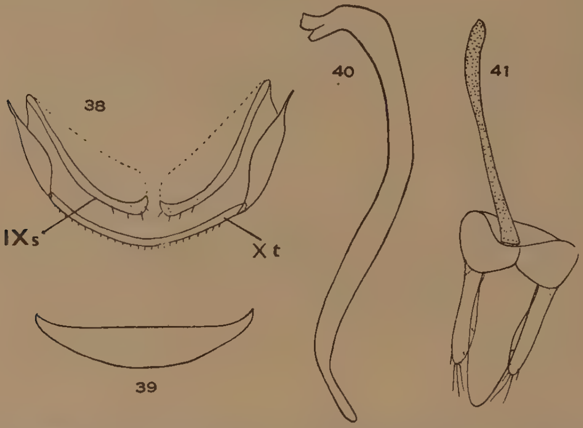
Stethorus pauperculus Weise (figs. 38–41).

Scymnus (subgenus *Stethorus*) *pauperculus* Weise, 1895, pp. 155–156.

Stethorus pauperculus Weise, 1900, p. 440.

Shortly oval, moderately widening in the middle and narrowing posteriorly; convex. Black, excepting the testaceous antennae, mouth-parts and legs which have sometimes reddish testaceous or piceous femora. Dorsal surface with greyish, moderately long, sparse and semi-erect pubescence. Head finely and sparsely punctate. Pronotum with fine and rather sparse punctures in the middle and coarse, navel-like and close punctures towards the lateral margins; elytral punctures rather coarse, less impressed, irregular in outline and close; surface between punctures on head and middle of pronotum smooth and shining but rather rugose on elytra. Underside with greyish, short, sparse and subdepressed pubescence and with rather close and coarse punctation, except on the median, rather flat, part of metasternum which has less coarse and sparse punctures and the elytral epipleurae and legs where the punctures are still finer and sparser. Femoral lines semicircular, complete, and extending a little beyond the middle to nearly two-thirds the length of the first abdominal sternite; the last abdominal sternite in the male (fig. 39) devoid of any emargination. Male genitalia: sipho (fig. 40), short, stout, narrowing gradually towards the apex, moderately curved near the base and obtusely bent in an opposite direction of the apical one-sixth; siphonal capsule, short, less distinctly marked, with the external branch semicircular, and the inner one very short and truncate; trab (fig. 41) long, nearly double the length of penis, dark, and spatulate at the distal end; basal plates wide; penis sub-triangular, with a slightly rounded apex and nearly twice as long as wide at the base; paramera short, nearly two-thirds the length of penis, filiform and with a number of short setae in the apical half. Female genitalia with the ninth sternite and tenth tergite strongly sclerotised only near the margin, each half of the former with four short setae near the apex.

Length 1·0–1·2 mm., width 0·8–0·9 mm.



Figs. 38–41.—*Stethorus pauperculus* Weise: (38) terminal segments of the female ×162; (39) sixth abdominal sternite of male ×75; (40) sipho ×162; (41) parts of male genitalia ×162.

Geographical Distribution:—In the original description, Weise (1895) gives three widely separated localities, namely " Konbir, Barway, Kurseong " in India. Later (1910 p. 232), he also records it from the Philippine Islands; this is probably due to incorrect identification, as superficially many species are very much alike. The true identity of the Philippine examples can be decided when more material from there is available. From my collecting experience and from the examples before me it appears that this is probably the most widely distributed and common species in India. Two examples of it from South Arabia have also been seen. The data are as follows:

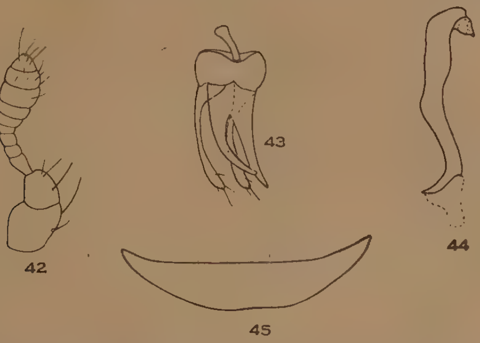
India: Madras Province: Coimbatore, –.vii.1910, predacious on mites on sorghum (*Y. R. Rao*); Coimbatore, –.v.1924 (*M. C. Cherian*). Mysore State: Bangalore, predacious on mites on *Andropogon*. Bombay Province: Nadiad, 3.xii.1914, feeding on mites; Poona, –.xi.1914 (*C. S. Misra*). Bihar Province: Pusa, –.iv.1909 (*D. P. Singh*); Pusa, 20.iv.1909, predacious on red spider on castor (*A. Murtaza*); Pusa, 13.xi.1909 (*H. M. Lefroy*); Pusa, 23.i.1914 on plantain (*D. P. Singh*); Pusa, 26.i.1914, on wing (*D. P. Singh*); Pusa, 5.vi.1935, on plantain leaf (*C. K. Samuel*). Delhi, 29.iii.1938, predacious on mites (*A. P. Kapur*). Punjab Province: Lyallpur, 23.iii.1935, predacious on mites (*M. Afzal Husain*).

ARABIA: West Aden Port, Al Huseini, near Lahej, 26.xi.1937 (*H. Scott* and *E. B. Britton*); Yemen, Wadi Thabad, 26.xii.1937, beaten from hedge (*H. Scott* and *E. B. Britton*).

REMARKS. Weise (1895, 1900) compares this species in detail with the description of *Stethorus* (*Pullus*) *rotundulus* (Motschulsky). From the original brief description given by Motschulsky, one may conclude that at least in coloration and size ($\frac{1}{2}$ line) *rotundulus* resembles *pauperculus*, yet Weise states that *rotundulus* is larger (1·5 mm.) than *pauperculus*. I have not seen examples of this genus from Ceylon and therefore prefer, for the present, to regard the two species as distinct. It may be pointed out here that later (1900, 1910) Weise regarded *rotundulus* as a synonym of *rotundatus* Motschulsky (1859, p. 70) on the assumption that the latter author probably confused the two names. In describing *rotundatus*, Motschulsky makes two important observations: (1) that the femoral lines are triangular and (2) that the larvae feed on *Pseudococcus* on coffee leaves; these observations indicate that *rotundatus* may actually be a *Scymnus*. I am, therefore, inclined to regard Motschulsky's two species as distinct.

Stethorus tetranychi, sp. n. (figs. 42–45).

Shortly oval, anterior margin of pronotum and the apices of elytra less narrow than in most other species, moderately convex. Black, excepting the uniformly pale yellow antennae, mouth-parts and legs. Dorsal surface with greyish, long, semi-erect moderately coarse and dense pubescence. Head minutely and sparsely punctate, club of antenna (fig. 42) rather robust and less tapering towards the apex. Pronotum moderately narrow anteriorly and at the anterior angles not quite so arched round the head as in *S. punctillum* Ws., or *S. pauperculus* Ws., punctation navel-like and not uniform, but fine in the middle, and coarse and close towards the lateral margins; elytral punctures irregular, coarse and usually confused in the basal two-thirds of the elytra and rather fine and distinct in the apical one-third; surface between punctures smooth and shining on head and pronotum, but less so on the elytra. Underside with greyish, moderately long, sparse and subdepressed pubescence; punctation moderately coarse and close except on the metasternum where the punctures are much coarser. Metasternum flat in the middle and sloping towards the lateral margins. Femoral lines wide, evenly rounded and extending to near the middle of the length of the first abdominal sternite; the sixth abdominal sternite in the male only weakly and broadly emarginate at the apex (fig. 45). Male genitalia: sipho



Figs. 42–45.—*Stethorus tetranychi*, sp. n.: (42) antennae × 162; (43) parts of male genitalia × 75; (44) sipho × 75; (45) sixth abdominal sternite of male × 75.

(fig. 44) short, stout, slightly narrowing towards the apex, obtusely bent between the middle and the apical one-fifth of the length ; siphonal capsule small and indistinct ; trab very short and club-like, basal plates (fig. 43) wide ; penis bifurcated into two sub-triangular tubes, from the apex to two-thirds of its length ; paramera slightly longer than penis, narrow in the middle, club-like towards the apex, bearing a few short setae.

Length 1·3 mm., width 1·0 mm.

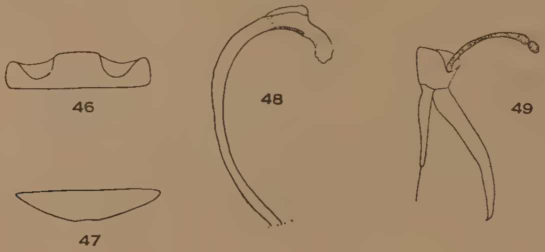
Type. In the British Museum ; India : Bengal, Dacca. Predacious on red spider (*Tetranychus* sp.) on jute (*G. M. Das*).

Paratypes. Two in the British Museum, three in the Indian Museum and three in the author's collection, with the same data as the type.

Remarks. The uniformly pale yellow colour of the antennae, mouth-parts and legs appears to be normal and not due to immaturity. The species is closer to *S. pauperculus* Ws., but can be distinguished by the differences in general shape, pubescence, punctation of the body, shape of the femoral lines and structure of the male genitalia.

Stethorus parcepunctatus, sp. n. (figs. 46–49).

Sub-hemispherical ; strongly convex. Black, excepting the testaceous antennae, mouth-parts, tibiae and tarsi and the reddish testaceous trochanter and femora. Dorsal surface with greyish, long, delicate and sparse pubescence. Head very finely and sparsely punctate ; pronotum with coarse and navel-like punctures which are sparse in the median part and close towards the lateral margins of the pronotum ; elytra coarsely, shallowly and sparsely punctate with the surface between the punctures smooth and nearly shining. Underside with greyish, short, sparse and subdepressed pubescence, and coarse, impressed and sparse punctation except on the prosternum and the legs which have finer punctures. Femoral lines (fig. 46) wide, semicircular, and extending a little beyond the middle of the segment ; last abdominal sternite (fig. 47) in male slightly emarginate at apex. Male genitalia : sipho (fig. 48) moderately long, narrow and curved proximally ; siphonal capsule short with a fairly wide and long inner branch, but without an outer branch ; trab (fig. 49) black, more than half the length of the penis, narrow, slightly curved, and knobbed at the distal end ; basal plates as long as wide ; penis elongate, tubular, narrowing from the base towards the pointed apex and slightly bent in the middle towards the paramera which are filiform, nearly half the length of the penis, and each provided with a long seta at the apex.



Figs. 46–49.—*Stethorus parcepunctatus*, sp. n. : (46) first abdominal sternite ×34 ; (47) sixth abdominal sternite of male ×75 ; (48) proximal part of sipho ×162 ; (49) parts of male genitalia ×75.

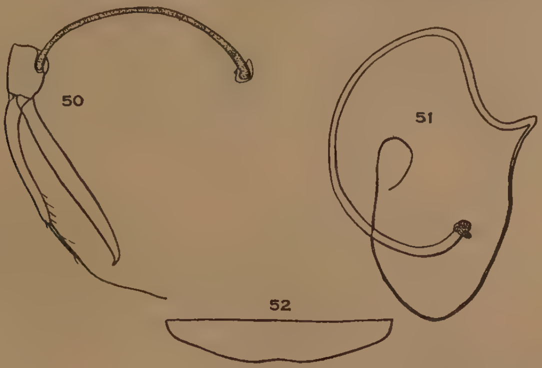
Length 1·0 mm., width 0·76 mm.

TYPE. In the British Museum; locality INDIA, Kanara (Andrewes' bequest). Another example with similar data was dissected and mounted on slides and card.

REMARKS. This species is easily distinguished from *S. pauperculus* Ws. by its sub-hemispherical and strongly convex shape, very sparse and long pubescence and sparse and distinct punctation.

Stethorus rani, sp. n. (figs. 50–52).

Shortly oval, convex. Black, excepting the pale testaceous antennae, mouthparts, apices of femora, tibiae and tarsi and the piceous to reddish-testaceous remaining parts of the legs. Dorsal surface with greyish, fairly long, semi-erect and sparse pubescence. Head minutely and sparsely punctate; pronotum with almost uniformly fine and close punctures, none of which is navel-like; elytral punctures slightly coarser, closer and much less impressed than those on pronotum. Underside with rather short, sparse and subdepressed pubescence and fine, impressed, and moderately close punctation except in the middle of the metasternum where the punctation is a little sparser. Femoral lines semicircular, complete and extending beyond the middle to nearly two-thirds the length of the segment. Last abdominal sternite in male (fig. 52) very weakly emarginate at apex. Male genitalia: sipho (fig. 51) very long, narrow, tapering towards the apex and irregularly curved; siphonal capsule very small, like a dark knob with irregular outline; trab (fig. 50) black, long, slender and slightly curved; basal plates nearly as long as wide, penis elongate, a little shorter than trab, tubular and tapering at the apex which is slightly bent towards the paramera; the latter two-thirds as long as penis, with four or five short setae at the apical one-third and a very long and stout seta at the extreme apex.



Figs. 50–52.—*Stethorus rani*, sp. n.: (50) parts of male genitalia ×75; (51) sipho ×75; (52) sixth abdominal sternite of male ×75.

Length 1·4 mm., width 1·1 mm.

TYPE. A male in the British Museum; locality INDIA, Ranikhet, Kumaon Hills, United Provinces (*H. G. Champion*).

PARATYPES. Four, in the British Museum (one dissected, the parts being mounted on a slide) with the same data as the type except one from Bodair, Haldwani, United Provinces (*H. G. Champion*).

REMARKS. This Himalayan species is distinguishable by the punctation of the pronotum and the structure of the male genitalia.

Stethorus aptus, sp. n.

Shortly oval, slightly narrowing posteriorly; moderately convex. Black, except the reddish testaceous anterior part of clypeus, the testaceous antennae, mouth-parts, tibiae, tarsi and apices of the femora and the piceous remaining parts of the legs. Dorsal surface with greyish, moderately long, sparse and semi-erect pubescence. Head finely and sparsely punctate; pronotum with coarse, navel-like punctures which are closer together towards the lateral margins than in the middle; elytral punctures coarse, impressed and fairly close to one another; the surface between the punctures less smooth than on the head and pronotum. Underside with greyish, short, sparse and subdepressed pubescence; punctation on the whole fine and moderately close except on the meso- and metasternum where the punctures are coarse, impressed and close. Femoral lines widely rounded, complete and extending to near the middle of the length of the segment. Genitalia unknown.

Length 1·3–1·4 mm., width 1·0–1·1 mm.

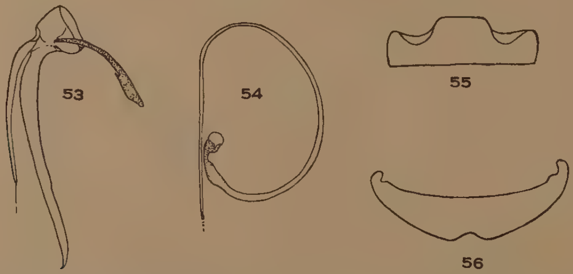
Type. In the British Museum; China, Tygosan Island, Chusan Archipelago, (near East Coast) (*Walker*).

Paratypes. Two, in the British Museum; China, Nimrod (II) (near East Coast) (*Walker*); China (near East Coast) (*Bowring*).

Remarks. This species has the appearance of *S. punctillum* Ws., from which it differs in its coarser and comparatively sparser punctures on the elytra, and also by the reddish testaceous clypeus. In the latter character it resembles *S. gilvifrons* (Muls.) which, however, has very fine elytral punctures and wider femoral lines which do not reach the middle of the length of the first abdominal sternite.

Stethorus siphonulus, sp. n. (figs. 53–56).

Shortly oval, widest in the middle, slightly narrower posteriorly, convex. Black, except the testaceous clypeus, antennae, mouth-parts, apical half of femora and tarsi and the castaneous basal half of femora. Dorsal surface with greyish, moderately long, sparse and semi-erect pubescence. Head finely and sparsely punctate; pronotum with rather uniformly coarse, navel-like and moderately close punctures; elytral punctures slightly finer, closer but less impressed than those on pronotum, giving a rippled appearance to the entire surface of the elytra. Underside with greyish, short and sparse pubescence; the meso- and metasternum fairly coarsely and closely punctate, the remaining parts less coarsely punctate. Femoral lines evenly curved and extending as far as one-third the length of the segment (fig. 55).



Figs. 53–56.—*Stethorus siphonulus*, sp. n.: (53) parts of male genitalia ×75; (54) sipho ×75; (55) first abdominal sternite ×34; (56) sixth abdominal sternite of male ×75.

Last abdominal sternite in male (fig. 56) distinctly emarginate at apex. Male genitalia: sipho (fig. 54) thin, long and whip-like; siphonal capsule slightly swollen near the base, constricted in the middle and almost rounded towards the apex which is weakly chitinised and is not as dark as the rest of the capsule; trab dark (fig. 53), moderately long and thin except at the apical one-fourth which is expanded; penis tubular, nearly twice the length of the trab, narrowed and curved at the apex; paramera slender, slightly longer than the trab and each with a short seta at the apex.

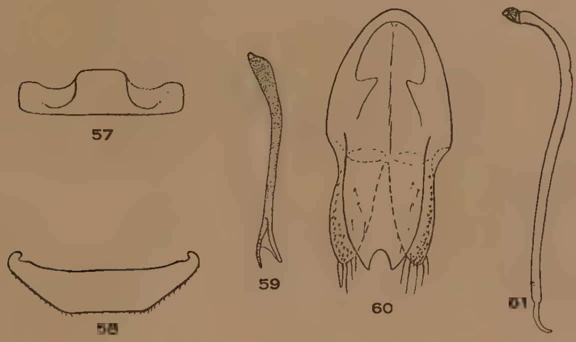
Length 1·2 mm., width 0·9 mm.

Type. A male in the British Museum; Malaya, Penang (Browning's Bequest).

Paratype. A male (dissected) in the British Museum; Malaya, Kuala Lumpur, 21.vii.1939.

Stethorus truncatus, sp. n. (figs. 57–61).

Shortly oval, strongly convex. Black, except the testaceous antennae, mouthparts, tibiae and tarsi, and the reddish-testaceous remaining parts of the legs. Dorsal surface with greyish, short, sparse and rather subdepressed pubescence. Head with moderately fine and sparse punctures; pronotum with fine, impressed and sparse punctures except near the anterior angles where they are closer; elytral punctures similarly fine and sparse but a little less impressed; surface between punctures smooth and shining. Underside with a pubescence similar to that on the dorsal surface and with the punctation usually moderately fine and close. Femoral lines (fig. 57) wide, semicircular, but with the external half of each so faint as to appear incomplete; the lines reaching two-thirds the length of the segment. Last abdominal sternite (fig. 58) in male broadly truncate posteriorly. Male genitalia: sipho (fig. 61) fairly stout and moderately long, curved slightly near the proximal end and straight for most of its length, but the apical one-eighth narrower and curved slightly in the opposite direction; siphonal capsule short, knob-like and dark; trab (fig. 59) dark, elongate, bifurcate at the proximal and slightly expanded at the distal end; basal plates (fig. 60) conical in outline, very much enlarged, larger than penis; penis two-thirds as broad as long, evenly curved at the lateral margins and narrower towards the apex which has a deep semicircular emargination; paramera elongate, as long as penis, rounded at the apices which bear about six long setae and also having many small setae over their outer (upper) surfaces.



Figs. 57–61.—*Stethorus truncatus*, sp. n.: (57) first abdominal sternite ×34; (58) sixth abdominal sternite of male ×75; (59) the trabs ×75; (60) basal plates, paramera and penis ×162; (61) sipho ×75.

Length 1·2 mm., width 0·9 mm.

TYPE. In the British Museum; MALAYA, Kuala Lumpur, 21.viii.1939. One more example bearing the same data, dissected, the parts being mounted on a slide.

REMARKS. This species may be easily distinguished from *S. siphonulus* from Malaya by its coloration, punctation and pubescence and also by the structure of the male genitalia which is unique in that the basal plates are very well developed; by this last character and by the truncate last abdominal sternite and the apparently incomplete femoral lines, it is separable from all other species of the genus, forming a group by itself.

Stethorus vagans (Blackburn).

Scymnus vagans Blackburn, 1892, pp. 248, 250; Korschefsky, 1931, p. 149.

Stethorus vagans (Blackburn) Weise, 1908, p. 13.

Shortly oval, fairly wide in the middle and moderately convex. Black, excepting the narrow anterior margin of the clypeus, antennae, mouth-parts and legs; dorsal surface with greyish, long, rather stout, sparse, and semi-erect pubescence. Head closely and finely punctate; pronotum with the punctures fine and sparse in the middle and coarse, navel-like and close towards the lateral margins; elytral punctures coarse, less impressed, irregular in outline and moderately close, except towards the apical one-fourth of the elytra where they become a little finer and sparser; the space between the punctures somewhat shining but not smooth. Underside with greyish, short and sparse pubescence, punctation coarse, impressed and close, except on the prosternum where it is fine and close and on the legs where it is fine and sparse as usual. Femoral lines rather narrow, semicircular, complete and extending to three-fourths the length of the segment, the sixth abdominal sternite in the male with a deep triangular emargination which nearly subdivides the segment.

Length 1·00 mm., width 0·8 mm.

TYPE. A male in the British Museum; AUSTRALIA, Victoria (*Koebele*).

REMARKS. Very close to the next species (*nigripes*) from which it may be distinguished by the form of the body and the characters of coloration, punctation and pubescence. The fact that Korschefsky treated it as a species of *Scymnus* suggests that he was not aware of Weise's (1908) paper on the Australian species.

Stethorus nigripes, sp. n. (figs. 69–70).

Shortly oval, narrower than the preceding species and a little more convex. Black except the piceous antennae, mouth-parts and tarsi. Dorsal surface with greyish, rather short, sparse and subdepressed pubescence. Head finely and sparsely punctate; pronotum with uniform, rather fine and sparse punctures, which are impressed but not navel-like as in *S. vagans*; elytral punctures similar to those on pronotum except at the apex where they are shallower. Underside with the pubescence similar to that on the dorsal surface, punctation on the whole fine and fairly close, except on the middle of the metasternum which is nearly impunctate in the posterior two-thirds, but has oblique furrows (formed as if by the fusion of punctures) on the anterior one-third; femoral lines (fig. 69) rather narrow, semicircular, extending to two-thirds the length of the segment; shape of sixth abdominal sternite in male not known, but in the female the segment is entire posteriorly, each half of the ninth sternite sub-triangular (fig. 70).

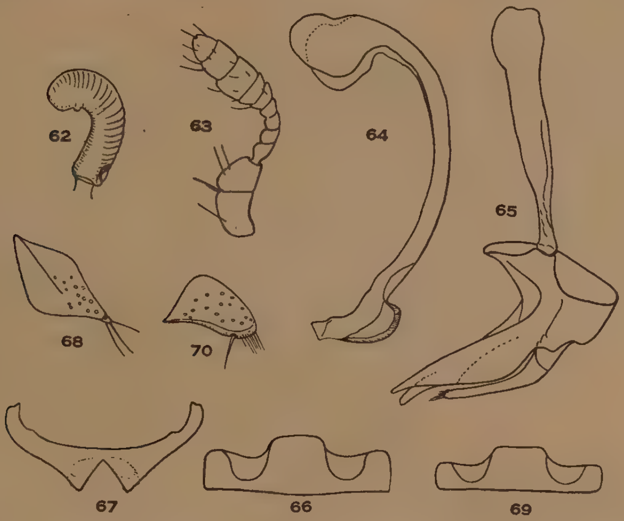
Length 1·0 mm., width 0·76 mm.

TYPE. A female in the British Museum; WEST AUSTRALIA, Albany (*C. D. Willis*).

REMARKS. By the comparative narrowness of its body and the difference in coloration, this species appears to be quite distinct from *S. vagans* (Blackb.). The latter occurs in south-east Australia whereas *nigripes* comes from West Australia but since a sufficient number of examples of either species has not been available for examination, it is probable that several intermediate examples may be discovered in long series from various localities. Owing to the scarcity of material, the male genitalia could not be studied.

Stethorus bifidus, sp. n. (figs. 62–68).

Shortly oval, moderately wide in the middle and fairly convex. Black, excepting the testaceous antennae and first two tarsi of each leg, the piceo-testaceous mouthparts and the rather piceous tibiae. Dorsal surface with greyish, long, delicate, sparse and sub-erect pubescence. Head with fine and moderately close punctures, antennae with a fusiform club ; pronotum with fine, navel-like and sparse punctures except towards the anterior angles where the punctures are closer ; elytral punctures coarse, impressed, irregular in outline and fairly close, but in the moderately shining surface between the coarse punctures there are fine and sparse ones which can only be seen under very high magnification. Underside with the pubescence rather shorter than, but otherwise similar to, that on the dorsal surface ; punctation on the whole coarse, impressed on the thoracic sternites and finer on the elytral epipleurae, abdominal sternites and legs, the punctures sparse on the middle of the metathorax and the first abdominal sternite ; femoral lines (fig. 66) moderately wide, semi-circular and extending to two-thirds the length of the segment ; sixth sternite in the male with a deep triangular emargination at the apex nearly bisecting the segment (fig. 67). Male genitalia : sipho (fig. 64) short, stout and slightly curved in the middle ; the distal end with a membranous swelling and truncate apex ; proximal part divided into two oval flaps ; trab (fig. 65) stout, long and spatulate ; basal plates wide ; penis hollow in the middle, gradually narrowing towards the apex which is subdivided



Figs. 62–68.—*Stethorus bifidus*, sp. n. : (62) spermatheca × 162 ; (63) antenna × 162 ; (64) sipho × 162 ; (65) parts of male genitalia × 162 ; (66) first abdominal sternite × 34 ; (67) sixth abdominal sternite of male × 75 ; (68) ninth sternite of female × 162.

Figs. 69–70.—*Stethorus nigripes*, sp. n. : (69) first abdominal sternite × 34 ; (70) ninth sternite of female × 162.

into two narrow lobes ; paramera filiform, short, about three-fourths the length of the penis, each with a cluster of short setae at the apex. Female with a well developed and strongly sclerotised spermatheca (fig. 62) which is moderately bent near the middle ; each half of ninth sternite (fig. 68) rhomboid in outline and with three stout and moderately long setae at the apex.

Length 1·0–1·15 mm., width 0·8–0·9 mm.

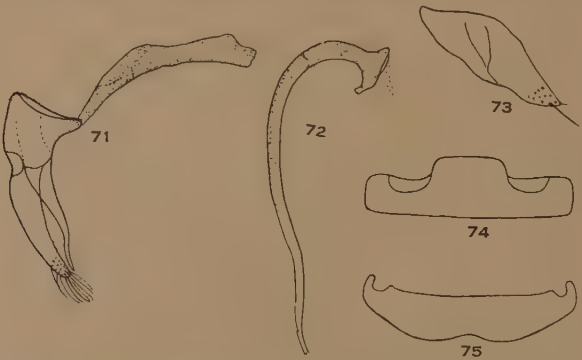
TYPE. A male in the British Museum ; NEW ZEALAND, Riwaka, predacious on *Bryobia* eggs (Mites). 18.i.1922 (*D. Miller*).

PARATYPES. In the British Museum ; four of both sexes with the same data as the type ; six of both sexes, New Zealand, predacious on red spider, –.i.1932, (*J. Muggeridge*) ; two females, New Zealand (*C. M. Wakefield*).

REMARKS. Cottier (1934) gave the biology of *Scymnus* sp. near *Scymnus minutulus* Brown. This is probably the same as *S. bifidus*. It is an important predator on mites and is more closely related to the Australian species than to those from other regions.

Stethorus fijiensis, sp. n. (figs. 71–75).

Shortly oval, rather strongly convex. Black, except the testaceous anterior half of the interocular space, antennae, mouth-parts and legs. Dorsal surface with dark-brown, long, moderately stout, sparse and slightly bent to sub-erect pubescence. Head with a few fine and impressed punctures ; pronotum throughout with coarse, impressed, navel-like and sparse punctures ; elytral punctures, coarse, impressed, subcircular in outline and moderately close ; surface between punctures on the entire dorsal surface smooth and shining. Underside with darkish, rather short and sparse pubescence ; mesosternum and anterior middle part of metasternum with coarse and sparse punctures, and the rest of the underside with fine, rather shallow and sparse punctures ; femoral lines (fig. 74) broadly curved and not extending to middle of length of segment ; last visible abdominal sternite (fig. 75) in the male with a wide but distinct emargination at the apex. Male genitalia : sipho (fig. 72) dark except in the apical one-third, short, widely curved proximally and straight distally, gradually narrowing from the base to the apex ; siphonal capsule indistinctly marked from the rest of sipho and with short inner and outer branches ; trab (fig. 71) large, very long and moderately broad ; penis elongate, sub-triangular, narrow and



Figs. 71–75.—*Stethorus fijiensis*, sp. n. : (71) parts of male genitalia ×75 ; (72) sipho ×75 ; (73) ninth sternite of female ×162 ; (74) first abdominal sternite ×34 ; (75) sixth abdominal sternite of male ×75.

pointed towards the apex and appearing sigmoid in side view; paramera stout, as long as penis, filiform and with long and closely set setae in the apical one-fourth. Each half of ninth sternite (fig. 73) in female rather sub-triangular, with two setae and several minute pores at the apex.

Length 1·4 mm., width 1·0 mm.

Type. A male in the British Museum; Fiji, Labasa, -.viii.1922. (*R. Veitch*).

Paratypes. In the British Museum; all from Fiji, four from Labasa, -.vii.1921 (*R. Veitch*); one from each of the following:—Lautoka, 12.ii.1920 (*W. Greenwood*); Cuvu, -.vi.1915 (*R. Veitch*); Suva, 12.vii.1921 (*H. W. Simmonds*); two examples dissected.

Remarks. By its dark-brown pubescence, this species is easily distinguished from all others of the genus. In the structure of the male genitalia also it is very distinct and forms a group of its own.

References.

Blackburn, T. (1892). Trans. roy. Soc. S. Aust., **15**, pp. 248–250.

Blattný, C. & Balaček, B. (1924). Pokusnické zprávy českého odboru rady zemědělské v Praze no. 3, 16 pp. Prague.

Borden, A. D. (1922). Mon. Bull. Calif. Dep. Agric., **11** (7), pp. 36–39.

Brèthes, J. (1924). An. Mus. nac. B. Aires, **33**, p. 167.

Casey, T. D. (1899). J. N.Y. ent. Soc., **7**, pp. 136–137.

Cherian, M. C. (1933). J. Asiat. Soc. Bengal, **27** (1931), pp. 141–147.

Cottier, W. (1934). N.Z. J. Sci. Tech., **16**, pp. 39–56. 657

Crotch, G. R. (1874). A revision of the coleopterous family Coccinellidae. 311 pp. London.

De Stefani, T. (1919). Boll. Studi inform., R. Giard. colon. Palermo, **5** (1–2), pp. 39–47. (R.A.E., (A) **9**, p. 56.)

Dobrzhansky, T. (1924). Ent. Mitt., **22**, pp. 18–27.

——. (1926). Bull. Acad. Sci. URSS, **20**, p. 1582.

Evans, J. W. (1943). Insect pests and their control. Dep. Agric. Tasmania, Hobart.

Garman, P. (1923). Bull. Conn. agric. Exp. Sta. no. 247 pp. 333–338.

Gilliatt, F. C. (1935). Canad. J. Res., (D) **13**, pp. 19–38.

Gmelin, J. F. (1789). Linn. Syst. nat., ed. 13, **1**, p. 1664.

Gyllenhal, L. (1837). Insecta Svecica, **2** (4), p. 195.

Hamilton, C. C. (1924). Bull. Maryland agric. Exp. Sta., no. 264, pp. 181–238.

Hargreaves, E. (1932). Rep. Dep. Agric. Sierra Leone, 1931, pp. 18–20.

Harold, E. von (1875). Coleopt. Hefte, **14**, p. 118.

Hauser, J. S. & Cutright, C. R. (1941). Proc. Ohio hort. Soc., **74**, pp. 26–34.

Herbst, J. F. W. (1797). Natursystem . . . . der Käfer, **7**, p. 346.

Illiger, J. C. W. (1798). Verzeichniss der Käfer Preussens, p. 413.

Jary, S. G. (1935). Ann. appl. Biol., **22**, pp. 538–548.

Korschefsky, R. (1931). Coccinellidae I.—Coleopt. Cat., pars **118.**

Listo, J., Listo, E. M. & Kanervo, V. (1939). Valt. Maatalousk. Julk., no. 99, 143 pp. (R.A.E., (A) **30**, p. 576.)

McGregor, E. A. (1913). Circ. U.S. Bur. Ent., no. 172, 22 pp.

—— & McDonough, F. L. (1917). Bull. U.S. Dep. Agric., no. 416, 72 pp.

Mader, L. (1924). Bestim.-Tab. europ. Col., **44**, p. 15.

Martelli, G. M. (1937). Riv. Patol. veg., **27** (1–2), repr. 12 pp.

Massee, A. M. (1940). Rep. E. Malling Res. Sta. 1939, **27**, pp. 70–73.

Menozzi, C. (1934). Industr. saccarif. ital., **27** (1), repr. 6 pp.

Motschulsky, V. (1858). Étud. Ent., **7**, p. 170.

——. (1866). Bull. Soc. Nat. Moscou, **39**, p. 426.

Moznette, C. F. (1919). Florida Bugg., **3** (3), pp. 45–48. (R.A.E., (A) **8**, p. 213.)

——. (1922). Bull. U.S. Dep. Agric., no. 1035, 15 pp.

Müller, O. F. (1776). Zoologiae Danicae prodromus . . . Havniae, p. 65.

Mulsant, M. E. (1850). Ann. Soc. Agric. Lyon, (2) **2**, 1104 pp.

Newcomer, E. J. & Yothers, M. A. (1929). Tech. Bull. U.S. Dep. Agric., no. 89, 69 pp.

Nunenmacher, F. W. (1937). Pan-Pacif. Ent., **13**, pp. 182–183.

Parker, W. B. (1913). Bull. U.S. Bur. Ent., no. 111, 43 pp.

Quayle, H. J. (1913). J. econ. Ent., **6**, pp. 85–88.

Radzievskaya, S. (1931). Za khlopkov. Nezavisim., no. 6–7, pp. 75–81.

Ross, W. S. & Robinson, W. (1922). 52nd Rep. ent. Soc. Ont., 1921, pp. 33–42.

Roubal, J. (1912). Ent. Mitt., **9**, p. 79.

Schaufuss, C. (1916). *In* Calwer, C. G. Käferbuch, ed. 6, **1**, p. 564.

Schilder, F. A. & Schilder, M. (1928). Arb. biol. Reichsanst., **16**, pp. 213–282.

Sicard, A. (1909). Ann. Soc. ent. Fr., **78**, pp. 146, 148.

Speyer, E. R. (1935). Rep. exp. Res. Sta. Cheshunt, **20** (1934), pp. 70–78.

Swezey, O. H. (1923). Hawaii. Plant. Rec., **27**, pp. 4–7.

Takahashi, R. (1938). Bull. Govt. Res. Inst. Formosa, no. 144, 10 pp.

Uspenskiĭ, F. M. (1937). Trud. sredneaz. Sta. Zashch. Rast., no. 2 pp. 3–25. Tashkent. (R.A.E., (A) **27**, pp. 625–626.)

van der Helm, G. W. (1935). Tijdschr. PlZiekt., **41**, pp. 313–315.

Vanwijngaerden, G. (1934). Agricultura, Louvain, **37**, pp. 296–304.

Webster, R. L. (1942). Bull. Wash. agric. Exp. Sta., no. 425 (52nd Rep. 1941–42), pp. 35–41.

Weise, J. (1879). Z. Ent., Breslau, **7**, pp. 140, 148.

——. (1885). Bestim.-Tab. europ. Col., ed. 2, **2**, p. 65.

——. (1891). Cat. Col. Europ., p. 781.

——. (1895). Ann. Soc. ent. Belg., **39**, p. 155.

——. (1899). Arch. Naturgesch., **65** (1), p. 64.

——. (1900). Dtsch. ent. Z., **1900**, p. 440.

——. (1908). Faun. S. W. Aust., **2** (1), p. 13.

——. (1910). Philipp. J. Sci., (D) **5**, p. 232.

Wollaston, T. V. (1854). Insecta Maderensia, 634 pp. London.

SOME ASPECTS OF THE ECOLOGY OF THE TICK, *IXODES RICINUS*, L., IN WALES.

By Don R. Arthur.

Department of Zoology, University of London, King's College.

Y.K

This paper is intended as a comprehensive ecological study of the distribution within a limited area (the county of Glamorgan) of the tick, *Ixodes ricinus*, L., and a general account of its distribution in Wales.

The distribution of Ixodid ticks in northern England and Scotland, has been the subject of detailed study (MacLeod, 1932 ; Milne, 1944), but no such study has hitherto been made for Wales. Presumably this omission is due to the scarcity of reliable records, for the sheep tick, *I. ricinus*, L., is certainly as important a disease vector in the lowlands of south Wales as it is in the highland pastures of northern Britain where the most important domestic host of this tick is undoubtedly the sheep. In Wales, on the other hand, it has been found that, especially in the south. cattle are more heavily infested than sheep (Edwards & Arthur, 1947) and the records also show that horses are subject to high tick incidence as are also goats at Llanuwchllyn, Merionethshire. Farm dogs, too, are liable to heavy infestation, but here one has to be careful not to confuse the sheep tick with the closely allied species, *I. hexagonus*, Leach, and *I. canisuga*, Johnston.

Examination of several thousands of ticks has shown that only one species, *I. ricinus*, infests sheep, cattle and horses in Wales. It is, therefore, rather surprising that Thompson (1936) does not refer to its occurrence in Glamorgan, although recording the two less frequent species, *I. hexagonus*, Leach, and *I. trianguliceps*, Birula (*tenuirostris*, Neumann), but unfortunately without stating the hosts or localities. Equally surprising is the fact that the sole reference by Nuttall, Warburton, Cooper and Robinson (1911) to *I. ricinus* in Wales is that, " Kossel and Weber, 1900, found this tick associated with redwater in North Wales ". The few records published are mainly from North and central Wales (MacLeod, 1939).

I. trianguliceps appears particularly to infest small mammals. The male of this species was first noted by Neumann on *Clethrionomys* (*Evotomys*) *glareolus* in Swansea, Glamorgan. The first Welsh record of *I. hexagonus* (Nuttall & others, 1911) was from the Aberystwyth area, and more recent records are given elsewhere (Arthur, 1947). In addition to these species, *I. uriae*, White (*putus*, Pickard-Cambridge), *I. canisuga*, Johnson, *I. unicavatus*, Neumann, and *I. vespertilionis*, Koch, have been noted. There are two records of *Dermacentor reticulatus*, F. (Walton, 1918), one of these was from a dog at Pen Dinas, Aberystwyth, Cardiganshire, and the second was seen in clothing at Llanfihangel-y-creuddyn, also near Aberystwyth. In addition a male specimen of *Haemaphysalis* has been recovered from a tortoise in the Cardiff district.

The data in the present paper have been based on the occurrence of adult female ticks on domestic stock (for sampling method, see Edwards & Arthur, 1947) and of all stages recovered from grassland by the ' blanket ' method (MacLeod, 1932 ; Milne, 1943).

Micro-ecological Studies in the County of Glamorgan.

Surface geology must to a large extent determine soil types, drainage and vegetation, and in Glamorgan it is of a complex character on account of the great variation of rock types and the influence of alluvial and glacial deposits. The latter may be stiff clays, impeding drainage, or they may consist of sand and gravel yielding free drainage; intermittent phases of such admixtures are common. Furthermore, Glamorgan also has a variety of relief features, both in broad outline and in detail, and it is convenient to consider these under four main divisions (fig. 1).

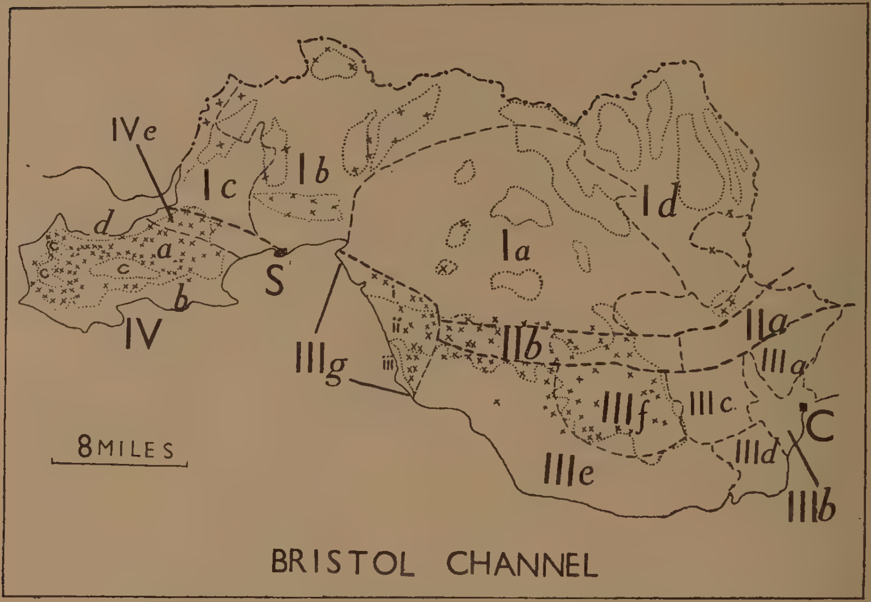


Fig. 1.—The distribution of *I. ricinus* in the County of Glamorgan. *Denotes infested holding; dotted lines the boundary of known infested pastures.

(i) Blaenau Morgannwg (Coalfield upland).
(ii) Border ridges of the coalfield.
(iii) Bro Morgannwg (Coastal regions).
(iv) The Gower Peninsula.

I. *Blaenau Morgannwg.*

This is a high plateau, 1,000 2,000 feet, (Ia) with ridges and valleys on either side (Ib and Id); much of it is occupied by the Pennant sandstone series of dull, grey-green grits, sandstones and subordinate shales. The eroded shales occupy areas below the general level of the plateau surface and the sandstones give rise to dry, thin, sandy soils, deficient in lime and with the *Festuca-Agrostis* plant association as a dominant feature. *Molinia* and *Nardus* grow on the moister flat summits and in the hollows while, under poorer conditions of drainage, peat formation proceeds in association with cotton grass (*Eriophorum polystachyon*) and rush (*Juncus communis*). Bordering the *Molinia-Nardus* zone is the mountain fescue pasture with bracken fern, with or without gorse, and this is a transition to the pure *Agrostis* pastures. The Pennant sandstone uplands introduce steep-sided hills covered with woodland and rough pasture, whilst in the middle of the farmland (Ib) glacial gravels occur with widespread alluvium in the valleys. Uncultivated peaty areas are frequent in the lowlands.

In the east of the coalfield are the Caerphilly and Gelligaer-Llantwit synclines, in which glacial gravel has been interrupted in its southward journey. The deposit is coarse and has produced irregular ridges which enclose peat bogs and alluvial flats. Where such areas are consistently stocked with cattle, *I. ricinus* is abundant and widespread.

Area (I) is either ill drained naturally or has such poor soil that the grazings are "rough", except for the marginal land. Ticks are widely spread in this zone. In most of the larger valleys, flat terraces alternate with steep bluffs in a series down the profile. The terraces are almost always cultivated and the soil is loamy, whilst the steep slopes are generally uncultivated, or in a low grade of cultivation and consist of acid peaty soil. There is also an alternation of tick free and tick infested land, according to whether the sward produces a "mat" vegetation or otherwise.

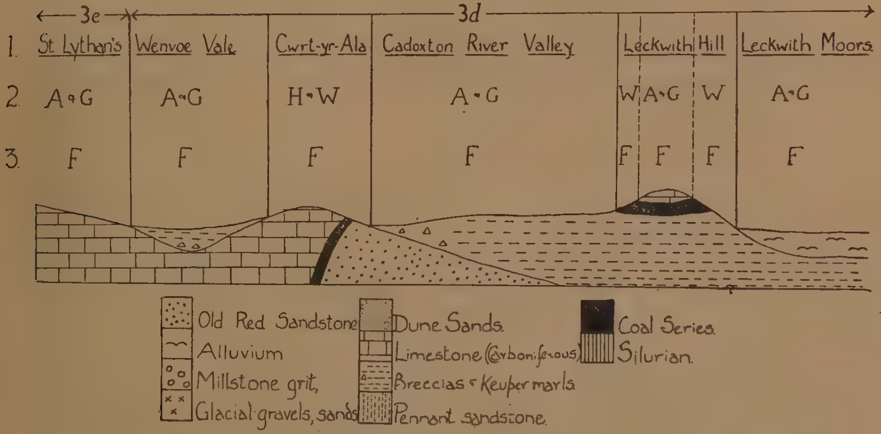
II. *Border ridges of the Coalfield.*

Bounding the coalfield uplands is a region of ridges and valleys. Here the rocks below the coal measures turn up sharply and expose to erosion a series of alternating hard and soft strata. These characters are fully developed in IIa where the drainage is free and intensive arable farming is practised. This sub-area is tick free. West of the Ely river (IIb) these ridges, except for the Millstone grit and Pennant sandstone, are covered by Mesozoic rocks. The millstone grit forms the ridges of Cefn Hirgoed and Cefn Cribwr, which are separated by a broad vale, on the lower coal measures, from the Pennant Sandstone escarpment that runs from Llantrissant to Port Talbot. Drift materials are a feature of IIb, with the exception of the aforementioned ridges and impeded drainage and poor soils are characteristic of the area. Consequently, the majority of the swards consist of coarse grasses, rush and sphagnum moss, with cotton grass in the hollows. This sub-area is a virulent source of tick infestation, the only non-infested grassland being along the Cefn-Hirgoed and Cefn-Cribwr ridges.

III. *Bro Morgannwg* (The coastal regions).

The plain of Llanishen (IIIa) is developed on red marls, the cornstones of the Old Red sandstones and the shales of the Silurian, with ridges of the two latter undulating above the general surface. This is a fertile, well cultivated sub-area and is tick free. The Leckwith Moors (IIIb), south-west of Cardiff, on the Taff and Ely flood plains, are artificially drained and free of ticks and form a centre for market gardening.

The Ely Valley (IIIc) is a drift-filled lowland yielding good agricultural land and is not infested.

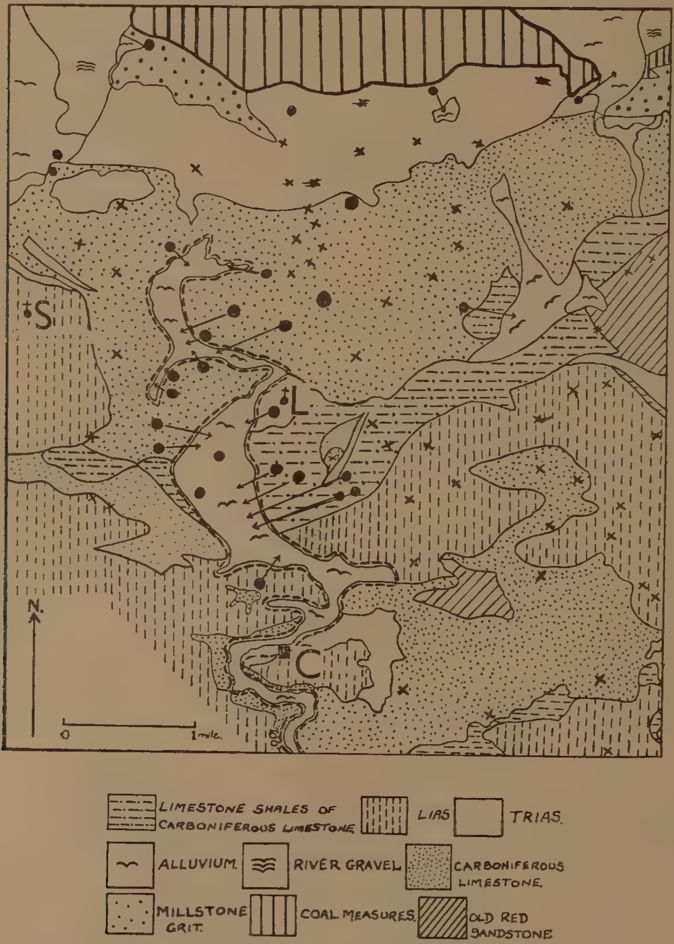


3e
3d
1.
St Lythan's
Wenvoe Vale
Cwrt-yr-Ala
Cadoxton River Valley
Leckwith Hill
Leckwith Moors
2
A·G
A·G
H·W
A·G
W
A·G
W
A·G
3.
F
F
F
F
F
F
F
F
Old Red Sandstone
Alluvium
Millstone grit,
Glacial gravels, sands
Dune Sands
Limestone (Carboniferous)
Breccias & Keuper marls
Pennant sandstone
Coal Series
Silurian

Fig. 2.—Section of the coastal region to show relation of sward to tick distribution. A, arable; G, grass; H, heath; W, woodland; F, tick-free pastures.

The basis of the subarea (IIId) is the plain of Keuper marls, forming the Wenvoe corridor and the Cadoxton valley, with Lias limestone outliers (at Leckwith and Lavernock) and the Carboniferous limestones of Cwrt-yr-ala. From fig. 2 it is seen that the region is intensively cultivated, with the proportion of "rough" cover to good grassland and arable small. Incidentally, the occurrence of heathland—dominated by heather (*Calluna vulgaris*)—in Glamorgan is not characteristic and its distribution is fortuitous. This sub-region is tick free.

The "proper" vale of Glamorgan (IIIe), consisting of Lias limestone and shales, and dissected by the Rivers Ewenny, Ogwr and Thaw is flat and well drained. The management of grassland reaches a high standard and the land is intensively


S
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1 mile.
LIMESTONE SHALES OF CARBONIFEROUS LIMESTONE
LIAS
TRIAS
ALLUVIUM
RIVER GRAVEL
CARBONIFEROUS LIMESTONE
MILLSTONE GRIT
COAL MEASURES
OLD RED SANDSTONE

Fig. 3.—The area north of Cowbridge, C, Cowbridge; L, Llansannor; S, St. Mary Hill. Dots, Tick-infested farms; Crosses, Tick-free farms. The arrows from the holdings indicate main pasture lands.

cultivated except for isolated patches of "downs". The natural drainage is free, and hitherto no ticks have been recorded from the good grassland areas or from the Downs.

The Ystradowen subarea (IIIf) is bounded by the Carboniferous limestone hills extending from Llanharry through St. Mary Hill to Cowbridge and thence to Bonvilston. It is a plain of glacial gravel fans and drumlins with islands of Carboniferous limestone, brownstones and quartz conglomerates. The drainage is very variable and there are extensive areas where the vegetation is rough. In the more retentive districts, as at Penlline moors (fig. 3) north of Cowbridge, this has given rise to semi-marshland conditions, in which large islands of heavy tick populations occur.

Between Kenfig and Briton-Ferry is the Margam coastal region (fig. 4) which is divisible into three main zones. IIIg(i) consists of boulder clay, glacial sands and gravels, with a gradual slope from north-east to south-west. Parts of it are cultivated, but towards the south-west much of it is neglected pastureland. The second area, IIIg(ii), is a broad area of flat, badly drained alluvial marshland, used for stock grazing. Protecting this lowland from invasion by high tides is a broad belt of sand dunes, IIIg(iii). Sub-area IIIg(ii) is a zone of heavy infestation, and there is evidence that this infestation is spreading to sub-area IIIg(i) particularly in the south east.

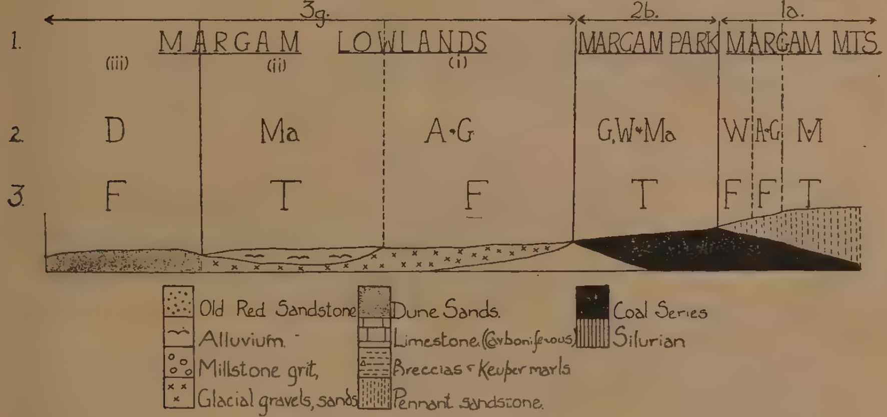

Fig. 4.—Section of the Margam area to show relation of sward to tick distribution. D, sand dune vegetation; Ma, marshland: A, arable; G, grass; W, woodland; M, Moorland; T, tick-infested pastures; F, tick-free pastures.

IV. *The Gower Peninsular* (figs. 1 & 5).

This is an elevated plain of Carboniferous limestone, covered by deposits of boulder clay, except where monadnocks rise above the surface. In some parts of the county the boulder clay is capable of cultivation (IVb), but in others it produces marshland, particularly where it is associated with carboniferous shales (IVa). This is seen in the large area of boggy moorland, north of the central east-west ridge of Cefn Bryn, which constitutes a continuous barrier of infertility on account of bad drainage. Sub-area IVb is well dissected by water courses, and the soils (derived from Pennant grits and Old Red sandstone conglomerates) are light loams with a high degree of fertility. The monadnocks (IVc) with their altitude, steep slopes and thin soils are moorland areas; the drainage is, however, relatively good, with a predominating amount of fescue in the sward, and it is heavily grazed by sheep.

A salt marsh on redistributed alluvium occurs on the north coast (IVd). The conditions found in IIb and IIIg(i) are repeated in IVe. Subareas IVa and IVe show a continuity of tick distribution whilst IVc has a localised distribution along the lower slopes.

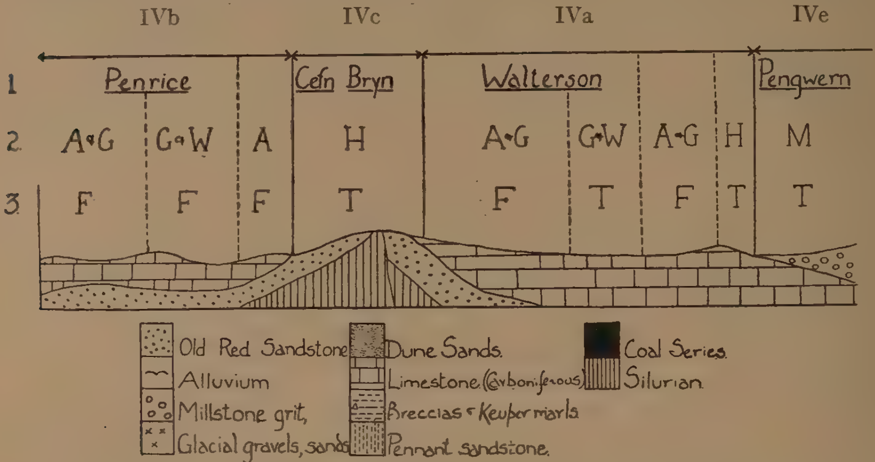

Fig. 5.—Section of the Gower Peninsula to show relation of sward to tick distribution. A, arable ; G, grass ; H, heath ; W, woodland ; M, moorland ; T, tick-infested pastures ; F, tick-free pastures.

The sheep tick infests roughly 200 of the 813 square miles comprising the county of Glamorgan and it is significant that the heaviest and most generally distributed infestations occur on land which lies below 400 feet ; but even within such limits the distribution is markedly discontinuous. The greatest extent of continuous tick distribution is found along the southern flank of the coal measures (IIb) and this is continued westwards into the north Gower Peninsula (IVe).

General Distribution in Wales.

The scarcity of records from Wales, already referred to, makes it desirable that a comprehensive survey should also be attempted of the distribution of *I. ricinus* throughout the Principality. While the information given here must present only a cross-section of actual occurrence, the maps and lists being necessarily incomplete, it does serve to show that the tick problem is an integral part of stock rearing in Wales and is worthy of more attention in the farming economy.

For purposes of the survey, Wales was divided into (i) north Wales (ii) central Wales and (iii) south Wales (fig. 6).

(i) *North Wales.* This has been taken as the plateau incorporating Snowdonia and the Berwyn range, which is bounded on the south by a line along the Dyfi valley to Lake Vyrnwy, along a tributary of the R. Severn and thence due north to the valley of the R. Dee. This incorporates the counties of Anglesey, Caernarvon, Denbigh, Flint, Merioneth and the northern extremity of Montgomeryshire.

The area is mountainous (exceeding 2,000 feet high) in parts and Snowdonia consists of old rocks, strengthened by hardened lavas. Neither the bare slopes nor the narrow valleys thus formed are able to support a rich agriculture. The infested areas of the north have accordingly been limited to those in which a covering of boulder clay, impeding drainage, has been deposited. In so far as this region is concerned, insufficient data are as yet available and conditions in Denbighshire, Flintshire and Anglesey have not been investigated thoroughly. The available data for the north are represented in Table I where reference is made to hosts from which ticks were recovered, type of seasonal activity (Edwards & Arthur, 1947) and types of vegetation from which nymphs were collected on infested holdings.

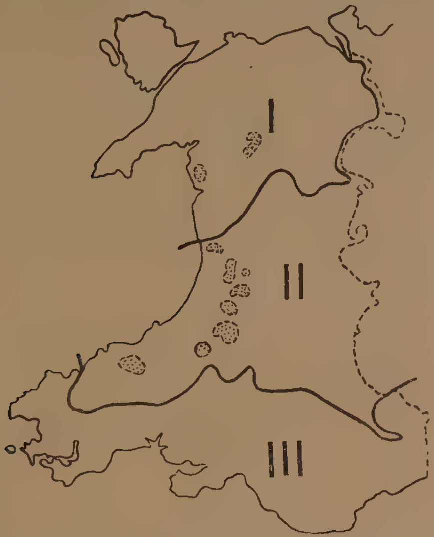

Fig. 6.—Map of Wales to show zones I, II and III discussed in the text. The shaded areas represent known areas of unimodal tick activity.

TABLE I.

County	District	No. of records	Hosts	Type of vegetation from which nymphs were recovered				Type of seasonal activity
				RG	B	H	R	
Caernarvonshire	Lleyn ...	12++	Sheep, cattle	—	10	1	1	Bimodal
	Gwyrfai ...	23++	,, ,,	—	18	—	5	,,
	Ogwen ...	2+	,, ,,	2	—	—	—	,,
	Nant Conway	21++	,, ,,	—	—	—	21	,,
	Eifionydd ...	23+	,, ,,	—	13	—	10	,,
Merionethshire	Llanuwchllyn	10++	,, ,, and goats	—	10	—	—	Unimodal
	Duffryn ...	15++	Sheep, cattle and horses	—	9	6	—	,,
Denbighshire Flintshire Anglesey		No data available						
Montgomeryshire	Llanfyllin ...	14++	Cattle	4	3	3	4	Bimodal
	Welshpool ...	5+	,,	1	—	—	4	,,
Totals ...		125		7	63	10	45	

The following abbreviations are used in Tables I, II and III.

1. Bimodal activity—two peaks of activity in a year :
   Unimodal activity—one peak of activity in a year.
2. + = Ticks present ; + + = very numerous.
3. RG = Rough grass ; B = Bracken ; H = Heather ; R = Rush.

(ii) *Central Wales.* The northern limit coincides with the southern boundary of the north Wales region. The southern boundary follows the gap of Usk, thence to Llandovery and westwards to Fishguard along the southern margin of the Cambrian mountains. With the exception of the narrow coastal plain of Cardigan this region is moorland, defined by Bowen (1943) as between 600 and 2,000 feet, composed of rocks of varying ages, but almost everywhere impervious to water. The covering of glacially formed clay also impedes the removal of surface water and consequently large stretches of this region have been turned into bog and ill-drained land, where *J. communis* and *E. polystachyon* are the dominant plants. Apart from the wet land, the vegetation is of rough grass and bracken providing poor sheep pasture. Despite this, there is a lack of " tick continuity ", in part due to the fact that much of it is not grazed by domestic stock. Further, as in Montgomeryshire and Cardiganshire, for example, where there is a great similarity in rock outcrop and soil cover, there are variations in the nature of the boulder clay coverings. It is these which, in Wales, largely influence the vegetation cover, as has been indicated previously in the Gower Peninsula (Glamorganshire). "Mat" formation is dependent initially on the vegetation and Milne (1944) has shown that the thicker the "mat" the denser the tick population. A summary of the results obtained in central Wales is presented in Table II.

TABLE II.

County	District	No. of records	Hosts	Types of vegetation from which nymphs were recovered				Type of seasonal activity
				RG	B	H	R	
Cardiganshire ...	Llarhystyd ...	4+	Cattle	—	4	—	—	?
	Taliesin-Talybont	3++	Cattle, sheep	—	1	—	2	Unimodal
	Llyfnant Valley	2++	,, ,,	—	2	—	—	,,
	Tregaron ...	6++	Cattle, sheep, horse, man (frequent)	—	6	—	—	,,
	Ciliau Aeron	1+	Cattle	1	—	—	—	?
	Pontrhyd-fendigaid	5++	Cattle, sheep, dogs (rare)	4	—	—	1	Unimodal
	Llanon ...	1+	Sheep	—	1	—	—	,,
	Llanybyther	3+	Cattle	—	3	—	—	Bimodal
	Lampeter ...	3++	Sheep, cattle	—	1	—	2	,,
	Llanfihangel-y-creuddyn	1+	Dogs	—	1	—	—	Unimodal
	Goginan ...	5++	Cattle, sheep, dogs, man	—	4	—	1	,,
	Pwllpeirian...	1+	Sheep, cattle	—	—	—	1	,,
Carmarthenshire (North)	Pumpsaint ...	1++	Sheep (lambs)	—	1	—	—	Bimodal
Montgomeryshire (South)	Llanerfyl ...	8+	Sheep	—	8	—	—	,,
	Carno ...	3+	,,	—	3	—	—	,,
	Llangurig ...	5+	Cattle, sheep	—	5	—	—	,,
Radnorshire ...	Disserth and Trecoed	10+	,, ,,	2	3	—	5	,,
	Llanfaredd ...	1+	Cattle	1	—	—	—	,,
	Lanydre ...	2+	,,	—	1	—	1	,,
Breconshire ...	—	0	—	—	—	—	—	—
Totals ...		65		8	44	0	13	B.M. 36 : U.M.24 ; ?5

(iii) *South Wales.* This region is composed of the South Wales coastal plain, together with the coalfield uplands and the Brecon Beacons. A large part of the region has been dealt with in some detail in the study of tick distribution in Glamorgan. Conditions on the northern edge of the coalfield are essentially similar to those seen along its southern margin in Glamorgan. The results are given in Table III. In contrast to the north and central regions tick infestation is far more prevalent at altitudes below 600 feet and cattle appear to be the host preferred, although horses in certain districts are subject to heavy attack. The information for this region is far more extensive than that available for the other regions, principally because of ease of access for collecting purposes.

TABLE III.

County	District	No. of records	Hosts	Vegetation belts from which nymphs were recovered				Type of seasonal activity
				RG	B	H	R	
Carmarthenshire (South)	Ammanford ...	12++	Cattle	—	—	—	12	Bimodal
	Llandebie ...	1++	,,	—	—	—	1	,,
	Penygroes ...	2+	,,	1	—	—	1	,,
	Maesybont ...	10++	,,	—	—	—	10	,,
	Porthyrhyd...	4++	,,	—	—	—	4	,,
	Tycroes ...	1+	,,	1	—	—	—	,,
	Llandilo ...	13++	Cattle, sheep and dogs	—	4	—	9	,,
Glamorganshire	Margam ...	43++	Rabbits, hares, deer, cattle, sheep and horses	2	—	—	41	,,
	Cowbridge ...	46++	Cattle, sheep and dogs	—	—	—	46	,,
	Coalfield Upland	38+	Cattle, sheep	—	20	—	18	,,
	Bridgend ...	28++	Cattle	—	—	—	28	,,
	Gower ...	78++	Cattle, sheep, and horses	3	7	1	67	,,
Pembrokeshire...	Haverfordwest	7++	Cattle	—	—	—	7	,,
	Lamphey ...	9++	,,	—	—	—	9	,,
	Gresselly ...	4++	,,	1	—	—	3	,,
	Maenclochog	8+	,,	—	3	—	5	,,
Monmouthshire	Pontypool ...	2+	,,	—	2	—	—	,,
Totals ...		306		8	36	1	261	B.M.306 ; U.M.O

Analysis of Factors influencing Distribution in Wales.

An analysis of the information collected from the three regions is tabulated below :—

Region	No. of records	Seasonal Activity		Vegetation belts from which nymphs were recovered			
		bimodal	unimodal	RG	B	H	R
North Wales	125	100	25	7	63	10	45
Central Wales	65	36	24*	8	44	0	13
South Wales	306	306	0	8	36	1	261
	496	442	49	23	143	11	319

*The seasonal activity of 5 of the records in doubt.

From this general survey it appears that the unimodal type of seasonal activity is absent in the south, occurs sporadically in the north and more frequently in the central region. Mention has been made elsewhere (Edwards & Arthur, 1947) of the seasonal activity curve with one peak, which commences towards the end of May, remains at a low level until July and then rises rapidly in August, thereafter declining in September and October. Activity of this type has been observed at Duffryn (Barmouth), Llanuwchllyn (Bala), and thence into Cardiganshire, along the western facing slopes, almost to Cardigan town itself (fig. 6). Typical conditions at Barmouth, Llanuwchllyn and Cardiganshire are illustrated in fig. 7, where records of the very heavy infestation of females attached to the hind quarters, groin and udder regions of cattle are shown.

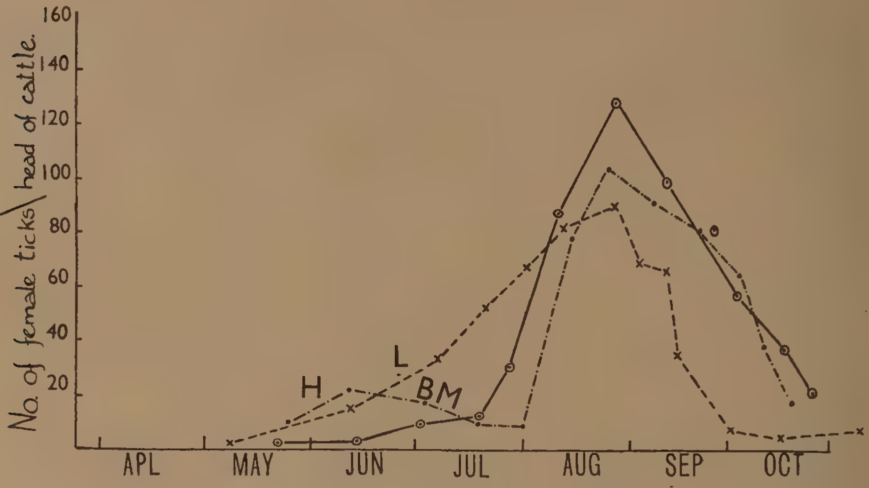

Fig. 7.—Typical tick activity curves in unimodal belt. H, Barmouth; L, Tregaron, Cardiganshire; BM, Llanuwchllyn—Bala.

An interesting feature of the results is the distribution of nymphal ticks relative to vegetation cover. In south Wales rush is more favoured than bracken, while rough grass and heather constitute only a small percentage (2·6 per cent.) of tick-infested land; in the central region bracken is infested 3·4 times more often than rush, while in the north the corresponding figure is 1·4, with rough grass and heather occupying some 13·5 per cent. of tick-infested land.

The analysis of pH values of the soil from some 187 tick-infested fields in the Principality has shown that they lie between 4·4 and 5·2. According to Milne (1944) there is " no association between the distribution of ticks and soil pH. . . . In short so far as fertility indices are concerned, there is no difference between tick-infested and uninfested hill land ". This is true for the actual data cited (Milne, 1944, Table I), but potentially the tick is capable of spreading to the " uninfested areas " either by transport, or if " uninfested " land adjoins infested land, by accretion. It would, therefore, seem that the only reason for non-infestation in uninfested hill land at the present time is the non-introduction of the tick. Milne (*op. cit.*) notes, in considering the relationship between *I. ricinus* to the environment, that " there is a consistent positive correlation between mat thickness and tick population density; the thicker the mat the denser the tick population irrespective of whether bracken or grass is the dominant ". The herbage which constitutes the " mat " is an accumulation of undecomposed and semi-decomposed root fibres and leafage. This is more prevalent on wet and acid soils where bacterial activity is interrupted and the decomposition of organic matter is arrested thereby encouraging the formation of "mat". Accordingly it is suggested that, as tick survival in grassland is correlated with the establishment of "mat" and as the latter is more rapidly developed under acid conditions, pH values do assume some measure of significance. This is in agreement with MacLeod's (1938) information, with Milne's figures and the present data. In this connection it is interesting to note the effects of lime on tick control in the field (Experimental Centre—Rudbaxton, Pembs.). The material employed was ground burnt limestone, containing 96 per cent. calcium oxide and applied at the rate of three tons per acre. The results obtained by various treatments applied on 13th June are indicated in Table IV.

TABLE IV.

Tick populations (based on mean of 5 drags/occasions).

Treatment	1945			1946										
	29.5	25.6	13.7	30.3	6.4	13.4	20.4	27.4	4.5	11.5	18.5	25.5	1.6	15.6
Disc harrow and Lime...	27·6	3·0	0·6	0	0	0	0	0	0	0	0	0	0	0·4
Pitchpole and Lime ...	15·4	1·6	0·4	0	0	0	0	0	0	0·4	0	0	0	0
Control...	21·4	18·4	9·8	0	0	3·4	2·0	4·4	7·6	1·6	12·0	15·0	13·4	3·4
Disc harrow only ...	11·6	14·8	4·0	0	0	0·8	0·8	1·4	0	2·4	1·0	3·6	0·6	2·0
Pitchpole only ...	18·4	15·0	4·2	0	0	0·4	1·2	2·4	1·8	0·6	3·2	5·8	2·4	3·4

The action of the lime was two-fold ; it served to reduce the degree of acidity and to destroy the mat. Its efficiency was comparable to benzene hexachloride and DDT, applied at the same time. The latter would presumably exercise only a direct acaricidal action and would, therefore, be only a temporary relief measure, while the action of lime in mat destruction and in reducing soil acidity would exercise a more lasting effect.

The Success of the Species relative to Habitat.

Three miles north-east of Lake Vyrnwy (alt. 1,000–1,300 ft.) and within the limits of a localised zone, four adjoining tracts of land with different vegetation cover were investigated for the presence of ticks. The area presents a V-shaped depression (fig. 8) and adjoining the northern slope is a tableland of limited extent, at a height of 1,300 feet. The area can be classified into the following categories :—

(A) the north facing slope, consisting of heather (*Erica tetralix*), winberry and *Agrostis canina*.

(B) the tableland ploughed from a rough bracken covering in 1944 and directly reseeded with a mixture of perennial ryegrass and clover.

(C) the valley bottom, considerably smaller in area than (A) or (B), where rush (*Juncus communis*), sphagnum moss and cotton grass (*Eriophorum polystachyon*) are the dominant species.

(D) the south facing slope, with a covering of bracken (*Pteris aquilina*) and underlying rough grasses, chiefly bents (*Molinia caerulea*).

All types of vegetation were equally accessible to stock and the populations on the different plots were sampled by the "blanket method". The drags on each zone, undertaken simultaneously, were equally distributed and it is thought that the data are sufficient to give the relative tick densities on the different types of vegetation, more particularly as they were carried out in the last week of April and the first fortnight in May.

Tick recovery was obtained in 70 per cent. of the drags in the rush type of vegetation as compared with 50 per cent. in heather, 50 per cent. in bracken and nil under ley conditions. The drags operated in the bracken, adjacent to the valley bottom, were more fruitful of tick recovery than the upper bracken slopes, as indicated by the ratio of recovered nymphs. The ratio of nymphal populations on lower to upper bracken was as 25 to 1. As the bracken growth in both was at the same stage of development, the effects of "blanket riding" discussed by Milne (1943) are eliminated. The manurial status, mat depth and relative tick densities of each plot as measured by the nymphal ground populations, are presented in Table V.

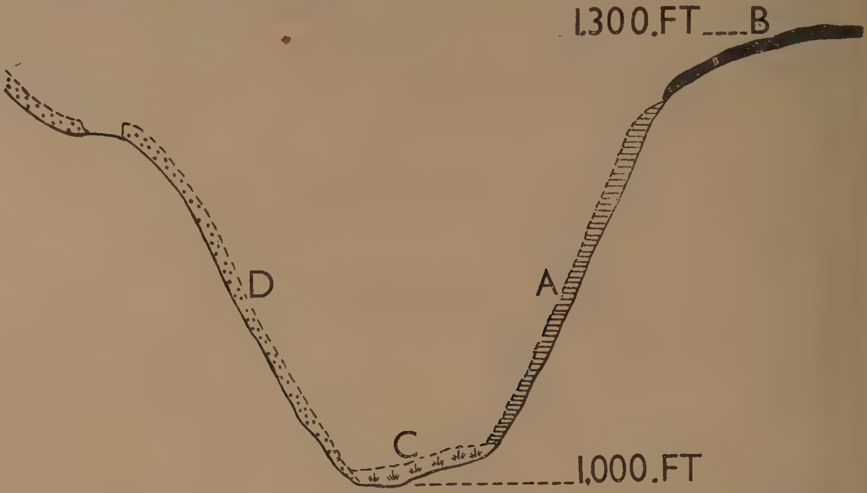

Fig. 8.—Section through a tick-infested zone at Llanfyllin showing distribution of vegetation types (see text). A, heather; B, ley; C, rush; D, bracken.

TABLE V.

Plot Index	Type	pH	Per cent. P_2O_5	Per cent. K_2O	Mat depth in ins.	Relative tick density (on 20 drags per 18 occasions)
A	Heather - winberry - Agrostis	4·57	0·009	0·020	0·7–1·3	9·0
B	Ley - perennial ryegrass and white clover	4·40	0·032	0·021	nil	nil
C	Rush - sphagnum moss - sedge	4·94	0·017	0·014	1·0–3·5	52·8
D	Bracken (i) Upper Belt ...	4·85	0·012	0·019	0·9–3·4	1·6
	(ii) Lower Belt ...	4·83	0·014	0·017	1·0–3·14	40·0

It is suggested that tick incidence was heavier at the foot of the bracken slope because of the more constant favourable humidity prevailing there, due to the more shaded conditions and the greater "run off" of water from the upper slopes. According to Milne (1944) the factors indicated are inadequate to account for this discrepancy within the same vegetation index, more especially as mat depths on both upper and lower bracken slopes are approximately equivalent (Table V). The case under discussion appears, therefore, to emphasise the importance of topographical location, in as far as it exercises an indirect effect on surface factors, apart from the determinant physical character of the vegetation. It is also interesting to note, *en passant*, that by readjusting the environment from "rough" to ley conditions there is a significant difference in tick populations, a feature which is discussed in some detail elsewhere (Report to the Agricultural Research Council, unpublished).

The different tick populations on adjoining vegetational types also presents an interesting problem along the northern littoral of the Gower Peninsula. Here a salt marsh is separated from a non-salt marsh by a narrow roadway. The salt marsh yields very little in the way of fresh grass or hay. Some 75 per cent. of the zone is covered principally by the sea rush (*Juncus maritimus*) with red fescue (*Festuca rubra*), buckshorn plantain (*Plantago coronopus*) and sea milkwort (*Glaux maritima*). On the non-salt marsh, *J. communis* is abundant and is supported by sphagnum moss as a basal type. The nymphal populations recovered, by blanketing, on the non-salt marshland are shown in Table VI.

TABLE VI.

Tick populations on non-salt marshland (based on 12 drags per occasion).

	Non-salt marsh			
	♂	♀	nymph	larva
3rd April ...	0	0	3	0
12th April ...	1	0	14	0
24th April ...	0	0	11	0
2nd May ...	0	0	24	0
9th May ...	0	1	12	1 cluster
16th May ...	0	0	27	2 clusters
23rd May ...	0	0	5	0
30th May ...	1	0	8	0
7th June ...	0	0	2	0

The blanket drags on the salt marsh yielded negative results and the examination of sheep in the area showed a complete absence of ticks. The examinations were undertaken simultaneously with those on the adjoining non-salt marshland, where positive results were obtained.

TABLE VII.

Sample	Depth of sample	Salt marsh		Non-salt marsh	
	inch	Per cent. Cl_2	Tick recovery	Per cent. Cl_2	Tick recovery
1	½	0·65	negative	0·05	positive
	1	0·71		0·02	
	1½	0·87		0·02	
2	½	0·72	negative	0·10	positive
	1	0·80		0·05	
	1½	0·96		0·06	
3	½	0·66	negative	0·11	positive
	1	0·74		0·07	
	1½	0·85		0·08	
4	½	0·84	negative	0·10	positive
	1	0·90		0·09	
	1½	0·95		0·07	

It is probable that ticks are absent from this salt marsh because of inadequate mat formation and relatively good drainage by the tidal channels which ramify through this sub-area. At the same time it must not be overlooked that the high concentration of salt may also have been a contributory factor. Samples of soil were taken with a four-inch core and the salt content (estimated as per cent. Cl_2) determined at three, half-inch, levels on both salt and non-salt marsh, the former being tick free, the latter tick infested. This data is presented in Table VII.

Influence of Habitat on Seasonal Activity.

Tick periodicity on cattle on certain farms in south Wales, even within a short distance of one another, fluctuates from the normal bimodal curve, and this factor is of some economic importance in determining the time to apply anti-tick treatment to stock. This is clearly seen by reference to fig. 9 where Farm L shows a curve of activity normal for the area and Farm N a delayed and irregular seasonal tick incidence. Both farms are situated in the Margam zone and are within less than half a mile of each other in a direct line. This irregular feature occurs annually and is in no way sporadic in its occurrence.

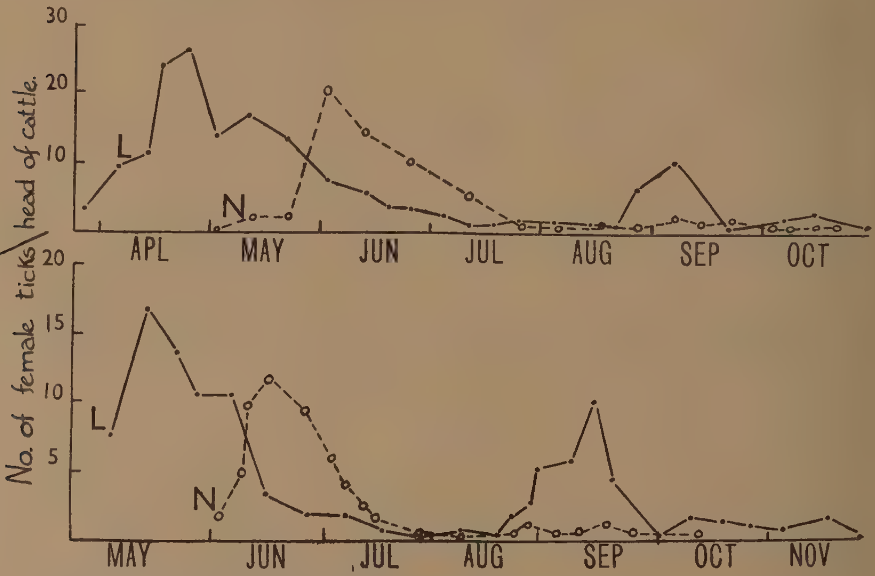

Fig. 9.—Bimodal activity of the tick in the Margam area (L) and delayed activity due to habitat considerations (N) in the same area, over a period of two seasons.

On holding L the cattle grazed five fields, which are characteristically of a rough sward of uniformly distributed rush. Here the seasonal activity follows the normal bimodal curve, commencing about mid-March, rising steeply in late April and May and thereafter declining to mid-July, being at a low level (generally less than two ticks per beast) in late July and August. Towards the end of the latter month and through September and October there is a recrudescence of activity which dies down in November and December. Throughout this period the cattle were continually on these pastures, except for the milking periods.

On holding N the pastures are mixed, good grassland being associated with "islands" of wet, marshy land, with shrub and copse growth which is excessively damp even in mid-summer. The seasonal incidence of ticks on cattle at N was erratic and in general occurred later than in L and did not attain the peak populations of the latter. The areas of infestation at L and N were kept under observation for a period of three years and it was found that, on a basis of nymphal counts, the ground populations were higher at L (Table VIII) and this may partly account for the numerical difference of ticks on cattle at the respective peak periods. The data show~~s~~ that, as far as *ground* activity is concerned, the periods of nymphal peaks are approximately coincident, whereas the information given in Fig. 9 indicates that, when population estimates are based on female ticks attached to cattle, this coincidence is not so.

TABLE VIII.

Ground populations of ticks (nymphs) on holdings L and N.
Figures based on the numbers collected in 5 drags on each occasion.

1st Year			2nd Year			3rd Year		
Date	Population		Date	Population		Date	Population	
	L	N		L	N		L	N
23rd April ...	27	13	20th March ...	0	0	8th April ...	0	1
28th April ...	13	5	27th March ...	0	0	17th April ...	0	2
5th May ...	18	10	6th April ...	8	2	24th April ...	0	1
12th May ...	24	9	11th April ...	10	4	1st May ...	10	3
19th May ...	2	0	17th April ...	115	21	8th May ...	28	13
25th May ...	3	0	24th April ...	31	12	15th May ...	113	34
31st May ...	7	0	27th April ...	45	21	22nd May ...	76	42
5th June ...	4	0	3rd May ...	34	15	29th May ...	12	1
8th June ...	2	1	11th May ...	17	8	5th June ...	15	12
15th June ...	3	1	15th May ...	19	7	12th June ...	3	5
22nd June ...	0	1	17th May ...	5	0	19th June ...	1	3
29th June ...	0	0	22nd May ...	7	1	26th June ...	0	0
10th July ...	0	0	12th June ...	2	0	2nd July ...	1	0
			19th June ...	1	0	8th July ...	0	0
			26th June ...	3	0	15th July ...	0	0
			2nd July ...	0	0	24th July ...	0	0
			7th July ...	0	0	30th July ...	0	1
			16th July ...	0	0	6th August ...	0	0
						13th August ...	0	0
Totals ...	103	40		297	91		259	118

It was observed that the highest tick populations occurred on the stock of holding N immediately following days of high temperature accompanied by bright sunshine, generally towards the latter part of May, June and July. (The absence of a meteorological station in the area prohibited the compilation of accurate temperature and sunshine records.) Associated with these photo-thermal changes there was a marked numerical and activity increase in the adults of *Hypoderma lineatum* and *H. bovis*, particularly of the form. The effects of the flight of the Diptera are to produce the characteristic "gadding" of cattle which induces them to seek shade conditions such as are provided by the copses in the islands of wet, marshy land. The examination of the hocks of the cattle some 2–3 days after "gadding" showed the presence of large numbers of warble fly eggs. Such an observation suggests most strongly that the "islands" are potential tick reservoirs. This would also partially account for the relatively lower population of ticks on cattle at N, even when the ground populations at L and N are equivalent, as the stock become subject to infestation at the latter end of the peak period.

This theory of isolated "suitable habitats" forming potential reservoirs was further examined at Farm C, Goginan, Aberystwyth, where discrepancies from the normal activity curve were likewise experienced.

At Farm C four fields were investigated for the presence of ticks by the blanket method. The details of the individual fields were as follows :—

Field 1. Directly reseeded with a mixture of perennial ryegrass and clover (1*a*). It is flanked on its southern boundary by a narrow belt of bracken and a growth of shrub vegetation which rarely exceeds four yards in width at any one point (1*b*). 20 drags were made in 1*a* and 8 in 1*b*.

Field 2. Well grazed in parts (2*a*) and adjoining a large copse on its western margin (2*b*). In the latter the ground is continually damp and has an extensive cover of sphagnum moss. The southern boundary is defined by a stream on the banks of which similar vegetation is to be found. 16 drags were made in 2*a* and 20 in 2*b*.

Field 3. An old established sward, well grazed and with a complete absence of rough herbage. 20 drags, distributed at random, were made.

Field 4. An old established well grazed pasture (4*a*) and a narrow woodland belt at its western extremity with an undergrowth of *Molinia* and bracken (4*b*). 20 drags were made in 4*a* and 16 in 4*b*.

An analysis of the results obtained from these data is presented in Table IX.

It is apparent that, as pointed out by MacLeod (1938), the problem resolves itself into two factors (*a*) tick spread and (*b*) tick survival. The actual spread of the tick to areas contiguous with infested pastures is determined by host movement, and the fate of such colonist ticks depends on their ability to survive in their new environment. Under the existing conditions at Farm C, it is extremely unlikely that the ticks from the damper rough vegetation would survive in the well grazed pastures. Nevertheless, the "islands" will remain potential areas of infestation, more especially if cattle are brought in from other tick infested land, as is the case on this particular farm.

TABLE IX.

Tick incidence (estimated as ground nymphal population) at Farm C, Goginan, Aberystwyth.

Field No.	Vegetation cover	No. of drags	Nymphal recovery/drag
1	(*a*) Directly reseeded	12	0
	(*b*) Bracken undergrowth	8	15·12
2	(*a*) Well grazed pasture	16	0·06
	(*b*) Copse-sphagnum moss abundant	20	15·3
3	Old established sward	20	0
4	(*a*) Well grazed pasture	20	0
	(*b*) Bracken & *Molinia*	16	12·7

The examination of ground populations in 'rough' land at Farm N showed that the bimodal activity characteristic of the belt is apparent, hence it would appear that the divergence from the normal activity curve on cattle bears a direct relation to their movement. This latter is determined by certain factors which cause the hosts to seek shade conditions in the vicinity of "tick reservoirs".

In practice, then, such "islands" of wet marshy land, in association with good pastures, should be fenced off and thus prevent cattle access to them. In addition, some protection may be afforded by providing shade and water in which cattle can stand. This is chiefly a matter of economics, but it is worth while noting that such precautions have been adopted with effect on some farms in the south Wales Province.

Summary.

The results presented in this paper apply to conditions in Wales. Although admittedly based on limited data, they show that of the nine species of ticks found in the Principality, only *Ixodes ricinus*, L. is found on sheep, cattle and horses.

It has been observed that the distribution of ticks throughout Wales is influenced by superficial soil deposits: studies in north, central and south Wales have shown, however, that the vegetation index from which ticks are recovered is variable. Analysis of soils from which ticks are recovered, gave a pH range of from 4·4 to 5·2, and the relationship between " mat " and soil acidity is discussed.

An investigation of four adjoining tracts of vegetationally different land—rush, bracken, heather-winberry and ley—showed that greater populations and a greater rate of activity were apparent in rush land. The significance of topographical location in relation to tick infestation even on land with the same vegetation index is indicated. A salt marsh adjoining an infested non-salt marsh was found to be free of ticks.

The occurrence of a unimodal activity curve is described for central and north Wales. Within a recognized bimodal activity belt irregularities of tick periodicity on cattle have been observed at some farms. This appears to be related to the nature of the sward, and, where good pasture occurs alongside " islands " of damp ground, such discrepancies are noted.

Such " islands " may harbour ticks, while the rest of the pasture is free. When, as the result of certain influences, cattle move to these " islands " they become infested. A meteorological condition is described which leads indirectly to such movement through the agency of the warble fly.

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The farmers of Wales have been most helpful in allowing me to examine their stock and to grant me all aid, often at considerable inconvenience to themselves, and I am most grateful for this service.

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References.

ARTHUR, D. R. (1947). Ent. mon. Mag., **83,** pp. 69–76.

BOWEN, E. G. (1943). Wales. A study in Geography and History, p. 23. Univ. Wales Pr.

EDWARDS, E. E. & ARTHUR, D. R. (1947). Parasitology, **38,** pp. 72–85.

MACLEOD, J. (1932). Parasitology, **24,** pp. 382–400.

——. (1938). Vet. ~~J.~~, **50,** pp. 1245–1250. Rec. / Rec.

——. (1939). Emp. J. Exp. Agric., **7,** pp. 97–110.

MILNE, A. (1943). Ann. appl. Biol., **30,** pp. 240–250.

——. (1944). Parasitology, **35,** pp. 186–196.

NUTTALL, G. H. F., WARBURTON, C., COOPER, W. F. & ROBINSON, L. E. (1911). Ticks, a Monograph of the Ixodoidea, pt. II, 105–348. Cambridge Univ. Pr.

THOMPSON, H. W. (1936). Glamorgan County History, **1,** Cardiff.

WALTON, C. L. (1918). Parasitology, **10,** pp. 206–231.

THE COMMON HOUSE-FLY, *MUSCA DOMESTICA*, L., AND ITS BEHAVIOUR TO TEMPERATURE AND HUMIDITY.*

By Sonti Dakshinamurty, M.B., B.S., B.S. Sc. (Madras),
D.P.H., D.T.M. & H., Ph.D. (London).

From the Department of Medical Entomology, London School of Hygiene and Tropical Medicine.

(Plate XXII.)

The study of the bionomics of the common house-fly has not received the attention it has deserved from Medical Entomologists, although it is the most widespread insect in the world, and the most familiar in view of its close association with man. Its taste for human food, drinks and discharges, habits of regurgitation, and deposition of faeces and infective material on human food are responsible for the rôle it plays in a variety of human diseases (Howard 1912 ; Graham-Smith 1913 ; Hewitt 1914). To it may be attributed a large amount of preventable sickness and mortality amongst those of all ages, and, in particular, infantile diarrhoea causing infantile marasmus in the tropics may be mentioned. The relation between the fly-borne diseases, such as infantile diarrhoea, enteric fevers and cholera, with such climatic factors as temperature, rainfall and humidity has been closely studied by several workers and by establishing their seasonal periodicity it has been possible to forecast epidemics (Roberts, 1906 ; Ainsworth, 1909 ; Niven, 1910 ; Hamer, 1908–10 ; Martin, 1913 ; Rogers, 1928 ; Russell and Sundararajan, 1928). But correlation between the incidence of these diseases and climatic factors is a correlation without causation. The present study of the behaviour of the house-fly in relation to climatic factors is an attempt to explain this correlation. The entomological aspects are considered in this paper, while the epidemiological considerations are dealt with elsewhere.

Breeding Methods and Technique.

Breeding media in nature and conditions favourable to breeding.

A constant supply of healthy flies for experimental purposes necessitates a proper choice of the breeding medium, as well as correct technique in rearing standard insects. In nature the house-fly prefers to breed in manure, but the commonest medium is household garbage. The medium selected for breeding is determined by the needs of the particular species of fly, as the female will deposit eggs only in material that will provide food as well as a home for the future maggot. Several writers have recorded that various species in different countries choose different media in which to lay their eggs (Newstead, 1909 ; Awati, 1919 ; Patton and Senior White, 1924 ; Thomsen, 1938). The species that choose animal manures and vegetable garbage are not so dangerous as transmitters of human diseases, as those that prefer human excrement. A careful analysis of these observations show that they depend on preferences due to physical factors such as temperature and moisture, or chemical factors such as decomposition and fermentation products that change the nutritive quality of products, or other factors that may attract the female fly to oviposit, that are not fully understood.

In a laboratory it is desirable to use a synthetic medium to produce a culture of standard flies. Variations in the quality of foods resulting in defective nutrition, or in the physical and chemical nature of the food restricting movement will result in the production of a small fly.

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*Part of a thesis for the Ph.D. Degree (Medical Entomology) of the University of London, in July, 1947 ; the complete thesis is available for consultation in the Library of the University.

The Behaviour to Humidity.

The study of the behaviour of the house-fly to humidity should be carried out in such a way that the final results can be interpreted quantitatively. Temperature increases metabolic functions and locomotor activity, and, as light may perhaps affect the latter, a constant temperature of 25°C. and a constant intensity of light at 30 foot candles to resemble daylight was adopted. A motor head-lamp with a parabolic reflector, fitted with a blue patent glass " lamplough " throws a parallel beam of light akin to daylight in quality (Plate XXII, fig. 1). Preliminary studies showed that a range of 20 per cent. R. H.* was suitable for the perception of humidity range by the flies, and for the sharp gradient of humidity essential for accuracy in the experimental chamber. Four sets of gradients were used—A (20–40 per cent.) ; B (40–60 per cent.) ; C (60–80 per cent.) ; and D (80–100 per cent.). With these gradients of relative humidity, constant temperature and light, the flies were kept in a " crawling " stage of activity which, for reasons stated later, is essential to enable them to choose the condition they prefer.

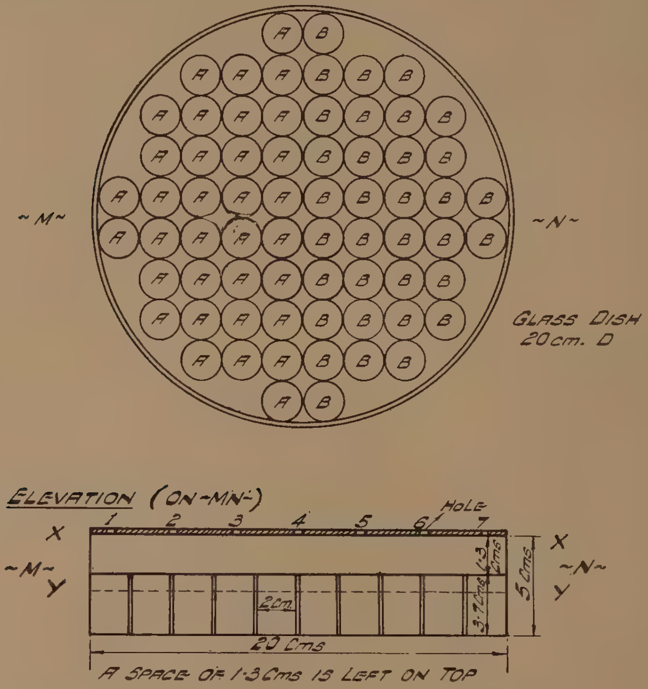

Fig. 1.—Apparatus for the study of humidity gradient experiments.

AA.	Capsules containing one R.H. mixture.
BB.	Capsules containing another R.H. mixture.
X.	Glass slab with seven holes to measure the humidity gradient as obtained in fig. 3.
Y.	Embroidery ring fixing a muslin platform which forms the floor on which the flies crawl.

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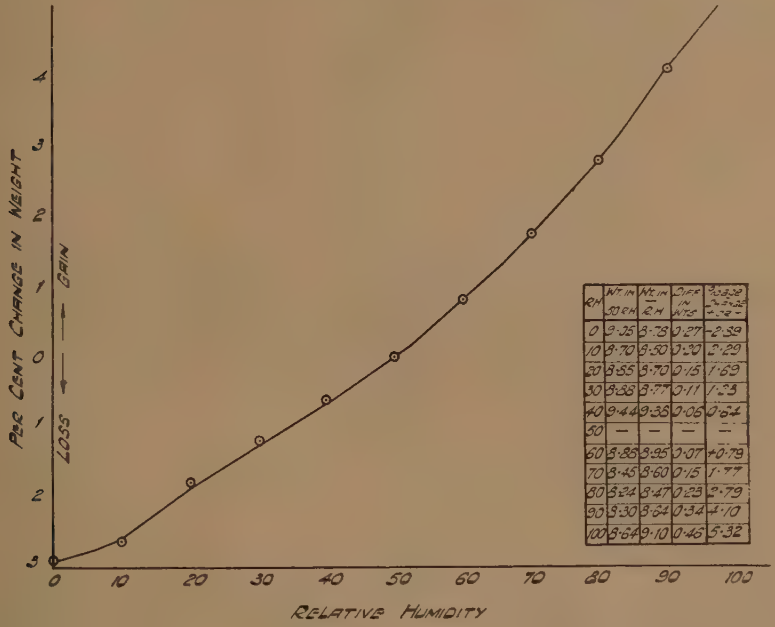
*The initials R. H. and S. D. are used throughout for relative humidity and saturation deficiency scales of humidity.

The Apparatus.

The control of humidity was effected by methods described by Buxton (1931*a*) and Buxton and Mellanby (1933) using sulphuric acid and water mixtures, whilst the apparatus was based on that devised by Gunn and Kennedy (1936) and Thomson (1938) with improvements to obtain more accurate results. A cylindrical dish 20 cm. internal diameter and 5 cm. deep was completely filled with about 70 thin glass capsules, each 3·7 cm. deep and 2 cm. diameter (fig. 1). Standard sulphuric acid-water mixtures were poured into these capsules up to a height of 3 cm., giving a 20 per cent. R.H. for A in one half of the dish and a 40 per cent. R.H. for B in the other half or similarly for any of the required combinations of A, B, C and D. A wooden embroidery ring with muslin stretched taut over it rested on the battery of capsules, and formed a floor for the flies. A circular glass slab about 22 cm. in diameter was placed on the top and formed the roof of a space 1·2 cm. deep in which the flies moved about. The glass slab had seven holes bored in it, each 1 cm. in diameter, the central hole being for the introduction of the flies and the others for tiny paper hygrometers calibrated as described by Mellanby (1933) (figs. 2 and 3).

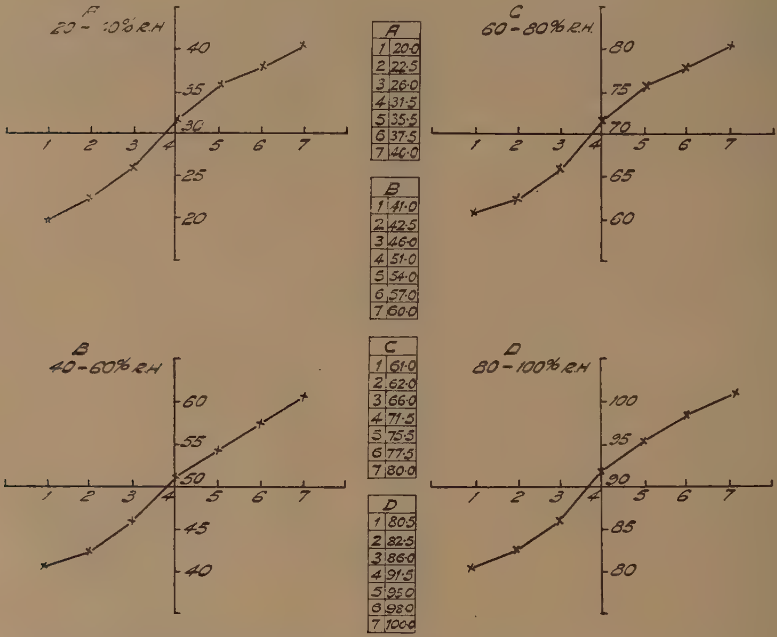


RH	WT. IN 50 RH	WT. IN — R.H	DIFF IN WTS	[illegible]
0	9·35	8·78	0·27	−2·89
10	8·70	8·50	0·20	2·29
20	8·85	8·70	0·15	1·69
30	8·88	8·77	0·11	1·23
40	9·44	9·38	0·06	0·64
50	—	—	—	—
60	8·86	8·95	0·07	+0·79
70	8·45	8·60	0·15	1·77
80	8·24	8·47	0·23	2·79
90	8·30	8·64	0·34	4·10
100	8·64	9·10	0·46	5·32

Fig. 2.—Calibration of the paper Hygrometer (Whatman No. 2). Kept at 50 per cent. R.H. and transferred to various humidities.

The apparatus was designed to give an enclosed space with a steep gradient of humidity, so that the two halves of the chamber approximated to the two humidity mixtures under study. This was achieved in the *vertical* plane by only just leaving sufficient height to enable the flies to crawl about, thus restricting movements of water vapour and in the *horizontal* plane by arranging batteries of capsules closely approximating to the two halves of the floor, giving the flies a common floor, but two humidity mixtures from which to choose.

A round, rather than a rectangular, chamber was adopted so as to avoid any corners. The number of flies selected was limited to ten, as a very large number in a small space would increase activity and the CO_2 content thereby upsetting the nature and degree of choice. In the case of a large number of flies, collisions would occur and an actively moving insect would often set in motion others that had made their choice of side and settled down. A large sample is more reliable for tests of statistical significance, but as the technique for small samples may be applied to numbers as small as ten, the " t " distribution curve becomes reliable for samples of ten and over, and so ten flies were chosen for each experiment.



A	
1	20·0
2	22·5
3	26·0
4	31·5
5	35·5
6	37·5
7	40·0

B	
1	41·0
2	42·5
3	46·0
4	51·0
5	54·0
6	57·0
7	60·0

C	
1	61·0
2	62·0
3	66·0
4	71·5
5	75·5
6	77·5
7	80·0

D	
1	80·5
2	82·5
3	86·0
4	91·5
5	95·0
6	98·0
7	100·0

Fig. 3.—Humidity gradient in the chamber. Points 1 to 7 in each graph are corresponding holes in glass cover in fig. 1.

Biological varieties may be considered under age and sex; conditioning also may influence the behaviour towards humidity. Flies 1–2 days old were used as they are active in nature at this age, and the two sexes were separately treated. The flies were all conditioned before use to ensure that the previous state of water balance did not upset their behaviour in choice of humidity; those conditioned at 30 per cent. R.H. are referred to as " Dry Flies," and those at 90 per cent. R.H. as " Wet Flies," while "Unconditioned" flies were used as controls. The temperature for conditioning was 25°C. for all experiments and the flies were kept at this temperature for half an hour. Thus they were differentiated into two sexes, three types of conditioned flies and four R.H. combinations, A, B, C and D; in all 24 treatments.

For each experiment the humidity mixture in the two halves of the chamber was arranged as described, the embroidery ring with fresh muslin placed *in situ* over the capsules, and the glass slab greased so as to make the chamber airtight and to enable equilibrium to be attained with the required humidity gradient.

Meanwhile flies 1–2 days old were sexed and conditioned at 30 per cent. or 90 per cent. R.H. in an incubator at 25°C. for half an hour. After the humidity gradient had been checked by paper hygrometers introduced through the seven holes and weighed quickly on a torsion balance and the holes sealed, flies were let in through the central aperture which was then closed. The motor head light was switched on and the number of flies on one or other half of the chamber counted every 2 minutes with a stop watch ; this was repeated ten times (Pl. XXII, fig. 2). The flies were discarded after each treatment and experiments were repeated with the two sexes, three types of fly and the four R.H. combinations.

Experimental Data and Statistical Analysis.

The mean results of ten experiments upon batches of ten flies in each of the 24 treatments for the flies choosing the *higher* humidity in each R.H. combination are given below in Table I. As the flies choose the *lower* humidity these figures are all less than 5 (Pl. XXII, fig. 2).

TABLE I.

	Dry Flies				Wet Flies				Unconditioned Flies			
	A	B	C	D	A	B	C	D	A	B	C	D
Males ...	3·5	3·5	4·5	2·2	2·8	2·0	3·6	3·2	3·5	3·6	4·4	4·5
Females	3·7	4·2	4·5	4·0	3·5	3·4	3·8	3·4	3·8	4·0	4·2	4·6

Experimental Data.

R.H. Gradients : A : 20–40 per cent. ; B : 40–60 per cent. ;
C : 60–80 per cent. ; D : 80–100 per cent.

These experiments raise four statistical problems which are discussed below :—

1. Whether in a chamber with the same humidity throughout, say 50 per cent. kept at a constant temperature of 25°C., the scatter of flies is by chance or by choice. Ten flies conditioned at 50 per cent. R.H. and 25°C. for half-hour were introduced into the chamber, and the numbers going to one half were counted a hundred times at two-minute intervals, and arranged in a frequency distribution. The difference between the expected and actual distribution was tested by the standard error of proportions, and by the χ^2 test, and it was found that the scatter was by chance and not by choice.

2. Whether in each of the 24 treatments above, the numbers going to one or the other side are significantly different from those going to the other half ; *i.e.*, is the choice significant ? The " t " test was applied in each case, and the values are given for each treatment in Table II below :—

TABLE II.

Test for Significance of Choice (" t " values of numbers in Table I).

	Dry Flies				Wet Flies				Unconditioned Flies			
	A	B	C	D	A	B	C	D	A	B	C	D
Males ...	** 5·8	** 10·5	* 2·4	** 9·3	** 9·3	** 14·9	** 9·0	** 7·5	** 5·2	** 5·5	* 3·0	* 2·4
Females	** 8·9	* 2·3	1·6	* 2·6	** 3·9	** 4·2	** 3·7	** 4·7	** 3·7	* 2·4	* 2·3	1·2

**Indicates significance at 1 per cent. level of P.
*Indicates significance at 5 per cent. level of P.

The choice in the case of wet flies is always significant at 1 per cent. level, as it is also in the case of females at either the 1 per cent. or 5 per cent. level with the exception of the dry flies under C, and the unconditioned flies under D.

3. Whether the *difference between the means* for the two sexes, the three types of conditioned flies, and the four R.H. combinations are significant ? The application of the " t " test, showed that the difference in the mean values of the Dry and Wet flies, and the difference in the mean values of Wet and Unconditioned flies are significant at 1 per cent. level, while the difference between the mean values of Dry and Unconditioned flies is significant at 5 per cent. level. The differences between the mean values of A and C, B and C, and C and D are significant, while the difference between the mean values of A and B, A and D, and B and D are not significant. In the case of males, C is significantly different from A, B and D, while in case of females, C is significantly different from A alone. The sex differences for Dry and Wet flies are significant, but not for Unconditioned flies.

4. By an analysis of variance, the main effects due to Sex, Conditioning and R.H. Combination were found significant at 1 per cent. level. The interactions for Conditions *versus* R.H. combination, and Sex *versus* Conditions *versus* R.H. combination, were alone significant.

The true mean values lie between the following limits with 95 per cent. probability:

Sex		Conditions		R.H. Combinations	
Male :	$3{\cdot}425 \pm 2(0{\cdot}0844)$	Dry :	$3{\cdot}763 \pm 2(0{\cdot}1034)$	A :	$3{\cdot}467 \pm 2(0{\cdot}1151)$
Female :	$3{\cdot}925 \pm 2(0{\cdot}0844)$	Wet :	$3{\cdot}213 \pm 2(0{\cdot}1034)$	B :	$3{\cdot}450 \pm 2(0{\cdot}1151)$
		Unc. :	$4{\cdot}075 \pm 2(0{\cdot}1034)$	C :	$4{\cdot}167 \pm 2(0{\cdot}1151)$
				D :	$3{\cdot}650 \pm 2(0{\cdot}1151)$

THE BEHAVIOUR TO TEMPERATURE.

The study of the behaviour of the house-fly to temperature requires suitable apparatus, and the experiments so designed that the final results can be interpreted quantitatively.

The initial difficulty was to design an apparatus in which insects could be exposed to a gradient of temperature without a gradient of humidity. Several workers in the past have appreciated this difficulty and have tried to get over it by biological or other devices. Gunn (1934) let in dry and saturated air into the cool and hot ends respectively of his temperature gradient apparatus to keep the relative humidity constant, and he regarded the resulting state as a response to a gradient of saturation deficiency. Mixtures of dry and moist air permit moisture to be deposited on the bodies of insects and as this evaporates in the dry air when they are towards the higher extremity of the temperature gradient, apart from the current of air itself upsetting their normal behaviour, it leads to a lowering of body temperature and introduces several complications in their thermotropism. Thomson (1938) realising that " whenever there is a temperature gradient, there must also be a gradient of relative humidity and saturation deficiency", got over the difficulty by a biological device. As Culicine mosquitos, with which he worked, failed to respond to differences of humidity up to 30 per cent. R.H., he worked with suitable small gradients of temperature below this figure and interpreted his final results as due to differences of temperature uninfluenced by differences of humidity. But the problem of designing an apparatus *with* a temperature gradient and *without* a gradient of humidity, that would be suitable for an insect such as the house-fly which can discern 20 per cent. R.H. differences, and others like locusts which can discern even 1 per cent. R.H. (Kennedy, 1937), remained unsolved.

With a gradient of temperature one may obtain the relative humidity or the saturation deficiency constant, but not both at the same time, for when one of these three is kept constant the other two can vary. Buxton (1932) and Mellanby (1935) have shown that when assessing the duration of life and water-loss during starvation in several insects, humidity is better expressed as saturation deficiency than relative humidity, as the saturation deficiency figures are directly proportional to the drying power of the air. The mechanism by which insects discern humidity is not understood at present but it may be that they may respond to humidity in one of two ways. Either by hairs on the antennae or elsewhere acting as hair hygrometers in which case they probably sense by the relative humidity scale, or more probably by evaporation or water loss from the body and by diffusion through the respiratory system, in which case they will respond to the saturation deficiency. This can be determined by noting any differences in the behaviour of flies in parallel experiments, using the same temperature gradient but with the relative humidity constant in one case and the saturation deficiency constant in the other. With this object in view, the entire series of experiments were duplicated with the temperature series, at 20–30°C. and 30–40°C., at constant R.H. and at constant S.D.

The Apparatus.

The apparatus in general outline was similar to that of Thomson (*loc. cit.*) but differed in several details (fig. 4). It consisted of a chamber, like the one already described under Humidity Gradient (fig. 1) but with different arrangements of the

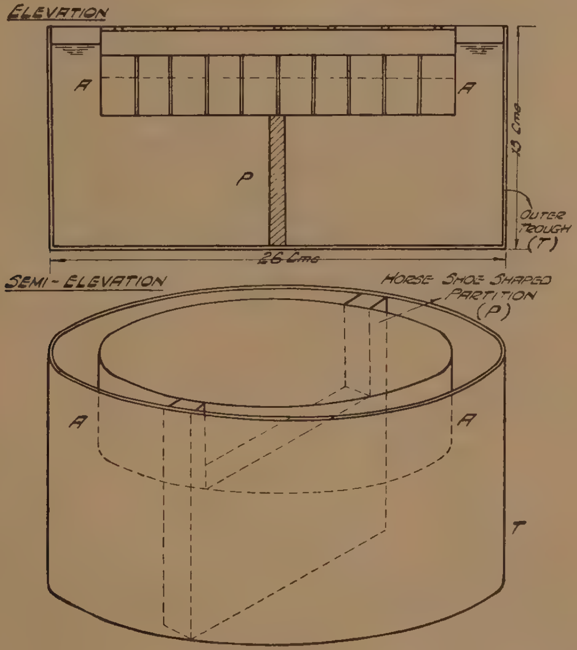


Fig. 4.—Apparatus for the study of temperature gradient experiments.

The chamber in fig. 1 is exactly the same as here (—M—N—). On either side of the Horse-shoe partition P, water is led through the outer chamber, T; one side 30°C., and the other side 20°C. or 40°C. to produce the gradients, 20–30°C. and 30–40°C.

sulphuric acid-water mixtures, as explained later. The chamber rested on a horse-shoe wooden partition separating the two halves of a metal basin where water at thermostatically controlled temperatures of 20°, 30° or 40°C. circulated from water baths to give the required temperature gradients of 20–30°C. and 30–40°C. The temperature control was therefore from the outer jacket, the humidity control from the chamber, and the light control from above as in the previous experiments (Pl. XXII, fig. 3).

It is necessary to explain in some detail how a gradient of temperature was obtained without a gradient of relative humidity in the one case or of saturation deficiency in the other. If an apparatus is arranged to give a temperature gradient of 30–20°C. with the same R.H. of 50 per cent. on both sides, a state of equilibrium will be attained in about half an hour but the relative humidity will not remain uniform, this will also form a gradient, say 70 per cent. at the cool end and 30 per cent. at the hot end owing to great evaporation of water vapour at the latter end condensing at the cool end. This prompted Gunn (*loc. cit.*) to put in dry and moist air to counteract the effect. A *falling* gradient of temperature will ultimately give a *rising* gradient

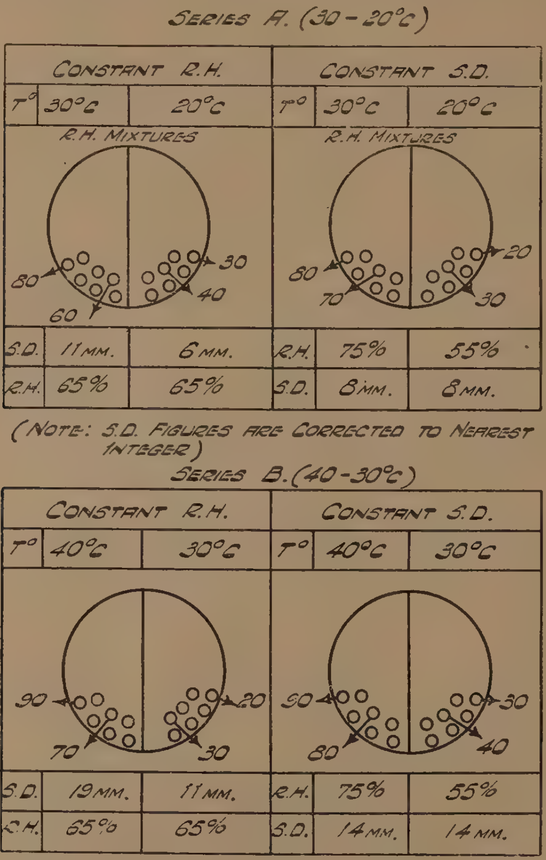

SERIES A. (30 - 20°C)
CONSTANT R.H.
CONSTANT S.D.
T° 30°C 20°C
T° 30°C 20°C
R.H. MIXTURES
R.H. MIXTURES
80
60
30
40
80
70
20
30
S.D. 11 MM. 6 MM.
R.H. 65% 65%
R.H. 75% 55%
S.D. 8 MM. 8 MM.
(NOTE: S.D. FIGURES ARE CORRECTED TO NEAREST INTEGER)
SERIES B. (40 - 30°C)
CONSTANT R.H.
CONSTANT S.D.
T° 40°C 30°C
T° 40°C 30°C
90
70
20
30
90
80
30
40
S.D. 19 MM. 11 MM.
R.H. 65% 65%
R.H. 75% 55%
S.D. 14 MM. 14 MM.

Fig. 5.—Temperature gradient at constant R.H. and S.D. Lay out of humidity gradients to counteract the effect of temperature on humidity.

of relative humidity. But, if we lay out to start with, a virtual *falling* gradient of relative humidity parallel with a *falling* gradient of temperature, when the apparatus attains equilibrium, we may obtain the *same* relative humidity. The virtual gradient of humidity is run under the muslin floor of the chamber in the sulphuric acid-water mixtures, so that in the space above the floor where the insects move about the same relative humidity is obtained. The equilibrium is dynamic and not static, and is obtained half an hour after the initial layout of the apparatus. The mixtures that must be used are primarily known by calculation, but later on more or less by trial and error.

If, for instance, a gradient of 30–20°C. at a constant R.H. of 65 per cent. is needed a gradient of R.H. is arranged from 80–60–40–30 per cent., so that it works out finally at 65 per cent. R.H. But the S.D. will have a gradient from 11 mm. to 6 mm. ; these S.D. figures correspond to 30°C. and 65 per cent. R.H., and 20°C. and 65 per cent. R.H. Again, if a temperature gradient of 30–20°C. at a constant S.D. of 8 mm., is required it will be seen from the tables that an R.H. gradient of 75–55 per cent. will result. By trial and error, the R.H. mixtures to be used will be 80–70–30–20 per cent. in the capsules. Thus, with the same temperature gradient of 20–30°C., at constant R.H. there is a gradient in S.D., and at constant S.D. a gradient in R.H. The same principle applies to other temperature gradients, such as 30–40°C. (fig. 5 and Pl. XXII, fig. 4).

Quantitative Experiments.

At the beginning of the experiment, the flies were first sexed as young males and young females (1–2 days old) and " fertilized females " (over 7 days old), so that changes in behaviour amongst older flies, after the development of the gonads, could be noted. The apparatus was laid out as explained above, for 20–30° and 30–40°C., at constant R.H. and at constant S.D. After half hour when the apparatus attained equilibrium, the temperature and humidity were checked up by thermometers and paper hygrometers ; the latter give R.H. figures only, these being translated into S.D. figures for S.D. gradients. The three types of flies with the two temperature series and the two humidity scales give 12 treatments. Each treatment was replicated six times, and the numbers going to one or other half in the chamber counted ten times at intervals of every 2 minutes, a different sample of flies being used for each replication. The mean number of flies going towards 30°C. in both gradients 20–30°C. or 30–40°C. was counted, as it was found they move towards 30°C. in each case (Table VI).

TABLE III.

Analysis of Quantitative Experiments.

Temperature Gradient	Constant R.H. or S.D.	Gradient of R.H. or S.D.	Choice	Dry or Moist Air
20–30* C ...	Const. R.H. 65% ...	S.D. 6–11 mm.* ...	Higher S.D. ...	Dry Air
	Const. S.D. 8 mm....	R.H. 55–75%* ...	Higher R.H....	Moist Air
30*–40 C ...	Const. R.H. 65% ...	S.D.11*–19 mm. ...	Lower S.D. ...	Moist Air
	Const. S.D. 14 mm.	R.H. 55*–75% ...	Lower R.H. ...	Dry Air

*Indicates choice in each gradient.

Choice towards 30°C. dominates humidity choice to dry or moist air whether expressed in the R.H. or the S.D. scale.

Qualitative Experiments.

(*a*) *Balanced Treatments.*

In order to confirm the previous findings that a temperature of 30°C. is favoured, a series of balanced treatments were arranged with young flies of both sexes in equal numbers. The temperature, R.H. and S.D. constants in these treatments were arranged so that they had a relation to each other ; the constants in one series being midway to the variables in the others in each gradient. Preferences to dry or moist air were recorded in each case (Table IV).

TABLE IV.

Analysis of Balanced Treatments.

(*a*) Temperature gradient 20–30°C.

Experiment	T°C.	R.H. Per cent.	S.D. mm.	Choice	Remarks
T. Constant ...	25	75–55*	6–11*	Lower R.H. Higher S.D.	Choose Dry Air
R.H. Constant	20–30*	65	6–11*	Higher T. Higher S.D.	Choose Dry Air
S.D. Constant	20–30*	55–75*	8	Higher T Higher R.H.	Choose Moist Air

(*b*) Temperature gradient 30–40°C.

Experiment	T°C.	R.H. Per cent.	S.D. mm.	Choice	Remarks
T. Constant ...	35	55*–75	19*–11	Lower R.H. Higher S.D.	Choose Dry Air
R.H. Constant	30*–40	65	11*–19	Lower T. Lower S.D.	Choose Moist Air
S.D. Constant	30*–40	55*–75	14	Lower T. Lower R.H.	Choose Dry Air

The S.D. figures are corrected to the nearest integer. * Indicates choice in each gradient.

The above experiments indicate that flies choose 30°C. irrespective of whether this temperature is associated with dry or moist air, and it made no difference whether this was represented by the R.H. or S.D. scale.

(*b*) *Treatments around* 30°C.

As the temperature preference towards 30°C. dominated the choice towards dry or moist air, experiments were designed to suppress this preference, and bring out the humidity preferences as found already in the humidity gradient experiments. A temperature gradient of 27–33°C. was selected and in one case constant R.H. was maintained and in the other constant S.D. so that the lower R.H. and the higher S.D. representing dry air corresponded to 27°C. and 33°C. respectively. Here the choice was towards drier air at both 27°C. or 33°C. (Table V).

TABLE V.
Treatments around 30°C. (33–27°C).

Temperature Gradient	Constants of Humidity	Gradients of Humidity	Choice	Remarks
33*–27°C.	Const. R.H. 60 Per cent.	15*–10·6 mm.	Higher T Higher S.D.	Choose Dry Air
33–27*°C.	Const S.D. 13 mm.	65–50* Per cent	Lower T Lower R.H.	Choose Dry Air

*Indicates choice in the gradient.

Experimental Data and Statistical Analysis.

The experimental data comprises twelve treatments, each treatment being replicated six times, and in each experiment ten observations were taken at intervals of 2 mins. The means of sixty observations are given in the table below (Table VI). In each case the numbers counted are those going towards 30°C., and this being the temperature preferred the figures are all over 5 in the Table.

TABLE VI.
Experimental Data (Quantitative Experiments).
(*a*) Series 20–30°C.

	Young Males	Young Females	Fertilised Females
Constant R.H.	6·2	5·6	6·0
Constant S.D.	5·2	7·1	6·0

(*b*) Series 30–40°C.

	Young Males	Young Females	Fertilised Females
Constant R.H.	5·2	5·4	6·0
Constant S.D.	6·0	6·0	5·8

The foregoing experiments raise four statistical problems.

1. Whether in a chamber at a uniform temperature of 30°C., and at a constant R.H. of 50 per cent., the distribution of flies is by chance or by choice. Ten flies were introduced into the chamber, and the numbers going to one side were counted one hundred times at intervals of 2 mins. and arranged in a frequency distribution. The difference between the numbers going to one side were tested by the standard error of proportions, and by the χ^2 test, and found not to differ significantly from an ideal chance distribution.

2. Whether in each of the 12 treatments at 30°C. in both series, the choice of side is significant (Table VII).

It will be seen from Table VII that with D.F.=59, all the "t" values are significant at 1 per cent. level.

TABLE VII.
Test for significance of choice.
("t" values of the figures given in Table VI.)

	Young males		Young females		Fertilised females	
	Const. R.H.	Const. S.D.	Const. R.H.	Const. S.D.	Const. R.H.	Const. S.D.
20–30°C.	8·02	7·2	4·85	22·1	7·99	5·22
30–40°C.	8·55	3·75	11·43	5·2	7·07	6·34

3. Whether the *differences between the means* for the two temperature series, the two humidity scales, and the three types of flies are significant. This is also ascertained from the "t" test, and the following results obtained. Taking each temperature series separately, the choice of males is significantly different for the two humidity scales, and of young females it is significant only in the lower temperature series, whilst fertilised females do not show any significant differences in either series. Considering both temperature series together, the choice between the two humidity scales is significant for young females, but not for males and fertilised females. The differences between the R.H. and S.D. scales are significantly different in the temperature 30–40°C., but not for the range 20–30°C. Lastly, the choice is significantly different when the humidity in the R.H. scale is constant with the S.D. scale operating, and not where the reverse is the case. It therefore appears probable that the humidity choice is dependent on the S.D. scale and not on the R.H. scale.

4. Whether by an analysis of variance, the three main effects for Temperature, Humidity and Types of fly are significant at 5 per cent. level. The interactions, Temperature *versus* Humidity, Temperature *versus* Types, Humidity *versus* Types are significant at 5 per cent. level; while the interaction, Temperature *versus* Humidity *versus* Types is significant at 1 per cent. level.

The true mean values lie between the following limits with 95 per cent. probability.

Sex
Young Males: 5·65±2 (0·1033)
Young Females: 6·05±2 (0·1033)
Fertilised Females: 5·95±2 (0·1033)

Temperature
Series: 20–30°C.: 6.02±2 (0·0843)
Series: 30–40°C.: 5·75±2 (0·0843)

Humidity
Const. R.H.: 5·73±2 (0·0843)
Const. S.D.: 6·02±2 (0·0843)

ACTIVITY IN RELATION TO TEMPERATURE AND HUMIDITY.

Reference has been made above to preliminary qualitative studies on the activity of flies in order to fix constants for temperature, humidity and light, in which they were kept in a "crawling" stage. In this stage, they make a deliberate and spontaneous choice, and the numbers counted on either side of the chamber give a cross section in space and time. The importance of this aspect of general behaviour suggested that a quantitative study of activity should be carried out in relation to temperature and humidity. Temperature increases the rate of development, and stimulates the metabolic rate and locomotor activity, which in turn increases numbers and activity. This series of events is familiar to everyone as increased "flies in circulation" in summer.

Nicholson (1934) studied the activity of blowflies at varying temperatures alone, keeping the humidity and light constant; while Key (1936) studied activity in relation to temperature, humidity and light. In nature, temperature and humidity changes cannot be divorced, and mosquitos are known to bite and become more active in moist air (Senior White, 1934); whilst flies are known to be active and very annoying in desert areas in the Middle East. Flies are markedly phototropic, and appear at night in the presence of strong light. The quality of light affects them as much as variations in quantity, as it is known that yellow and blue glasses admit light unattractive to flies (Austen, 1939). In all experiments, therefore, the quantity and quality of light was kept constant at 30 foot-candles resembling the sunlight to be found in living rooms on a bright summer afternoon.

Nicholson (*loc. cit.*) recognised five grades of activity in the case of *Lucilia*; viz., *Coma*, when insects lie on their backs and make no movements; *No Movement*, when they are on their legs without movement; *Movements other than Locomotion*, such as cleansing movements of the body; *Crawling*, slow steady walking, and *Flight*. This classification was adopted in all the experiments, and flies were kept in a crawling stage of activity, by adjusting light, temperature and humidity. The data under activity reveal that this has been done successfully by the absence of figures under the extreme grades of activity, *coma* and *flight* (Table VIII). Four combinations of temperature and humidity were taken, with high and low temperatures and high and low humidity; the former were fixed at 35°C. and 18°C., and the latter at 90 and 20 per cent. R.H. The object was to select marked contrasts between the high and low temperatures and humidity, without encroaching on the threshold values which would distress the flies. Six flies were put into the chamber in each treatment, and the procedure was to record at a glance the number of flies exhibiting each type of behaviour in the activity grades, and to make a large number of observations. The percentage frequencies were plotted as a column diagram which shows the relative differences between these treatments (fig. 6). The actual figures in the frequency distribution were subjected to a χ^2 test for the four combinations, as well as for high *vis-a-vis* low temperatures, and high *vis-a-vis* low humidities.

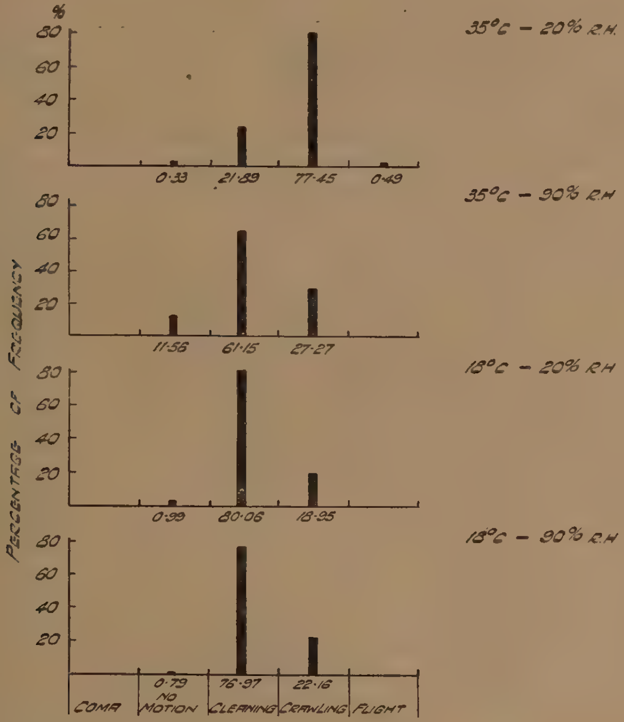


Fig. 6.—Activity of *Musca domestica* in relation to temperature and humidity.

Experimental Data and Statistical Analysis.

Four combinations with high and low temperatures and high and low humidity with five grades of activity are given below. The frequency distribution as well as the percentages of frequency are given below (Table VIII).

TABLE VIII.

Experimental data.

Temperature.
A : 35°C
B : 18°C.

Humidity.
X : 90 per cent. R.H.
Y : 20 per cent. R.H.

Activity	AY (35°C.—20%)		AX (35°C.—90)		BY (18°C.—20%)		BX (18°C.—90%)	
	FREQ	%	FREQ	%	FREQ	%	FREQ	%
Coma	—	—	—	—	—	—	—	—
No motion ...	2	0·33	56	11·56	4	0·99	5	0·79
Cleansing	134	21·89	296	61·15	320	80·06	485	76·97
Crawling	474	77·45	132	27·27	76	18·95	140	22·16
Flight	3	0·49	—	—	—	—	—	—
Total ...	613	100·00	484	100·00	400	100·00	630	100·00

The percentage of frequencies are represented in a column diagram (fig. 6). It is seen that the maximum activity is given with AY, the minimum with AX, while BY and BX are intermediate in value.

The frequency values *between* the different combinations are treated by the χ^2 test ; the χ^2 values are given in table below : The differences between the high and low temperatures and humidity are similarly treated (Table IX). It will be seen that with D.F.=9, all the χ^2 values are significant at 1 per cent. level of P. There is a significant difference between the several temperature and humidity combinations, and also between high and low temperatures and high and low humidities.

TABLE IX.

χ^2 values of grades of activity.

Grades of activity	AX-AY	BX-BY	AY-BY	AX-BX	A-B	X-Y
No motion	124·5	17·5	22·0	120·1	328·4	40·2
Cleansing	139·3	72·0	171·7	39·0	138·9	30·9
Crawling	40·6	44·8	64·4	21·0	92·3	12·5

DISCUSSION.

Physiological Considerations.

The results obtained in these experiments are discussed in relation to those of other workers on similar problems and different insects.

(*a*) *Behaviour to a gradient of humidity.*

Several workers have recorded the preference of various insects to a gradient of humidity at constant temperature. Kennedy (1937) found the African migratory locust preferred dry air in all parts of the humidity range at a constant temperature of 30°C. Gunn and Cosway (1938) found cockroaches if desiccated preferred dry air, and moist air if undesiccated, at a constant temperature of 25°C. Thomson (1938) found with *Culex fatigans* a strong reaction to avoid the high humidity range, and a weaker reaction to the lower humidity range. The findings with house-flies agree closely with those of locusts, but differ from the cockroach in that no reversible reaction was obtained with dry and wet flies, and to a smaller extent with the *Culex* at the high humidity ranges where it prefers the dry air.

The preference for dry air may be due to two causes ; either a preference to the environment containing dry air, or to the effects of dry air on the water balance of the insect. Metabolic rates being a function of temperature, the suggestion that low humidities cause greater increase in the rate of respiration and greater production of metabolic water may be ruled out (Mellanby, 1939). The site of water loss from insects was shown to be chiefly from the tracheal system (Mellanby, 1934) and certain insects such as the bed bug are indifferent to atmospheric humidity provided they get blood from time to time. House-flies, fed on sugar and water, may belong to the class of insects with efficient spiracle closing mechanism that conserve water from the rectum by drying of the faeces. The consistent results obtained with dry and wet flies throughout the entire range of humidity make this conjecture likely ; the flies might be closing the spiracles in the lower humidity range to conserve water, while opening them in the higher ranges to avoid developing a dropsical condition.

(*b*) *Behaviour to a gradient of temperature.*

While several workers have been interested in different problems relating to temperature, such as rearing, development, activity and thermal death points, there are few records relating to temperature preference at constant humidity, owing chiefly to the difficulty of constructing a suitable apparatus. Gunn (*loc. cit.*) experimented with *Blatta orientalis* and found it preferred a range of temperature of 20–30°C. independent of the humidity. Desiccated insects preferred a much lower range, 12–30°C. and this was explained as a preference shown by such insects to air with a lower evaporating rate, the apparatus being designed to give gradients of temperature and saturation deficiency with relative humidity constant. Thomson (*loc. cit.*) found *Culex fatigans*, in the range 10–30°C., avoided the higher temperatures of 25–30°C. and appeared to prefer a much lower temperature, perhaps round about 20°C.

The preference of house-flies for a temperature of 30°C. and dry air may be due to environmental temperature or the indirect effects of environmental temperature on body temperature. In the higher temperature series, 40–30°C., flies can remain at 40°C. by losing metabolic water at a rapid rate through widely open spiracles, requiring a greatly accelerated respiratory rate. The urge to open the spiracles on the one hand to take in more oxygen and to close them on the other to conserve metabolic water would probably result in a greater loss of water than of oxygen intake. This results in rapid desiccation, causing them to fly away from 40 towards 30°C. The question arises whether they would prefer 40°C. with dry air to 30°C. with moist air. At 30°C. there is not the same high metabolic rate as at 40°C., and consequently not the same rapid desiccation, and the insects are quieter at 30°C. and moist air than at 40°C. and dry air. Dry air is preferred to moist at 30°C. as it is easier to get rid of metabolic water, if necessary, and lower the body temperature below the environmental, whilst in moist air they may get dropsical. In the lower temperature series of 20–30°C., the flies choose 30°C. and dry air if possible, but 30°C. and moist air, if they must. At 20°C., metabolic and respiratory rates are low, and consequently there is no need to keep the spiracles open. At 20°C. and dry air they may get desiccated if their spiracles are open, whereas in moist air they would be more comfortable. Between 20°C. in moist air and 30°C. in dry air, they choose the latter as their metabolic rate increases, and excess metabolic water can be got rid of by opening the spiracles, thereby helping oxygen intake and loss of water in dry air, and keeping them fairly active all the time.

The two discrepancies in choice towards 30°C. in moist air in both ranges require further explanation. Flies at 40°C. may be killed directly by heat or indirectly by desiccation, the evaporative power of the air being higher at the higher temperature. By choosing 30°C. and 11 mm. S.D., in preference to 40°C. and 19 mm. S.D., they are choosing air at a lower evaporative rate. This is similar to the findings of Bacot and Martin (1924) who found fleas died more quickly at 32°C. than at 21°C. at the same S.D. of 10 mm. on account of the increased metabolic rate, opening of spiracles, and greater loss of water leading to desiccation. Again, flies at 20°C. and 55 per cent. R.H. going towards 30°C. and 75 per cent. R.H. are increasing their metabolic rate, and the excess metabolic water may be got rid of even at 75 per cent. by opening the spiracles, thus enabling the intratracheal air, which is saturated, to diffuse into air at 75 per cent., which is relatively drier.

(*c*) *Activity in relation to temperature and humidity.*

Key (1936) found that 3rd, 4th and 5th instar hoppers of the African Migratory Locusts were more active in dry than in moist air, while Kennedy (*loc. cit.*) got reverse results which he could not explain. Nicholson (1934) with *Lucilia* found increasing activity with rise of temperatures, but he did not observe their behaviour with combinations of temperature and humidity. The results with house-flies agree generally with those of Key and Nicholson (*loc. cit.*).

Activity grades may be considered on physiological grounds as corresponding gradations in the metabolic rates. The temperatures 35° and 18° were chosen with this in view as the higher is short of the thermal death point, and the lower is well beyond that where spontaneous movements would be possible, thereby affording a maximum contrast in activity. In the high temperature combinations with high or low humidity the flies wish to get rid of the excess metabolic water by opening their spiracles wide in dry air, and thereby lowering body temperature to a figure less than that of their environment. At 35°C. and 90 per cent. R.H. they produce excess metabolic water that they cannot get rid of owing to the humidity of the environment being near saturation point. On the other hand, at 35°C. and 20 per cent. R.H. they could easily get rid of this excess water in the dry air and this accounts for their maximum activity. In the low temperature combinations, the metabolic rate is low as it is a function of temperature and there is no need for the flies to open their spiracles, and so these combinations did not show any marked differences in activity. Flies may be taken as examples of insects with a perfect spiracle-closing mechanism, and dry or moist air combined with low temperature combinations does not affect them in the same way as high temperature combinations under which they must get rid of the excess moisture in dry air.

Interpretation of Behaviour in the Field in the Light of the Experimental Data.

(*a*) House-flies are generally active from sunrise to sunset which, apart from diurnal rhythm and phototactic behaviour, may be due to increasing temperature and decreasing humidity producing a peak in activity between 2–4 p.m., the hottest and driest hours of the day.

(*b*) On hot sunny days house-flies are mostly outdoor insects but under rainy conditions they are found indoors ; in both cases they move to warmer and drier places (Thomsen, 1934). However, if during rain it is warmer outside then they will remain outside (Peters, 1910). It is the higher temperature and lower humidity that sends them indoors, and not any direct effect of rain.

(*c*) In the tropics flies are generally less numerous in the coastal seaboards than inland ; the former are areas with moderate temperature and high humidity, while the latter show high temperature and low humidity. In India, cities similarly situated on the sea coast such as Madras, Calcutta and Bombay, with equable temperature and high humidity, have fewer flies than Delhi which is in the interior and is hot and dry. Flies near the deserts in the Middle East are a source of considerable annoyance because they alight on eyelids and lips in search of moisture and a few flies give the impression of a large population.

(*d*) In equatorial tropical countries one is struck by the remarkable freedom from house-flies, despite the fact that other insect life, such as mosquitos and cockroaches, is abundant. The climate of Malaya, for instance, is a combination of moderately high temperature and high humidity, the temperature being kept down and the humidity maintained by frequent showers amounting to an annual rainfall of over 100 inches distributed over 100 to 150 days. The reason for the scarcity of flies in such countries is perhaps due to a combination of unfavourable factors at various stages of its life-cycle. The eggs are washed from the surface of manure heaps by frequent showers and the breeding grounds are too wet. Frequent showers, alternating with bright sunshine at short intervals, render the manure heaps wet and cold from evaporation and arrest the heat of fermentation and decomposition so vital to the growth and activity of the maggots. The earth around the manure heap becomes sodden and saturated with moisture, if not puddled, thus preventing the emergence of the pupae which require a dry environment. Further, the young imago is unable to force its way through moisture laden soil with the same ease as through dry earth (Aldridge, 1907). Lastly, the adult insect finds an atmosphere of moderately high temperature and high humidity limiting to its activity and numbers are thereby reduced.

Factors that influence the Behaviour.

The house-fly like other domestic insects prefers to have feeding, breeding and resting places all within easy range, and the number and activity increase in proportion in accordance with the suitability of these and climatic conditions. The feeding and breeding places preferred by any species depend on the media available and the existing weather conditions at the time determine whether it rests outdoors or indoors, whilst its day and night movements and actual resting places are determined by its phototropic behaviour. Its prevalence reckoned as an index of the sanitary condition of the locality in conjunction with the state of activity, produces the "flies in circulation", and together with the number and crowding of the foci of infection, determine their importance as carriers of infectious diseases or as insects of mere nuisance value.

In the temperate zone where temperature is the dominant factor, the peak in numbers and activity is reached sometime in the summer. In the tropics with temperatures at no time unfavourable, and with a distinct rainy season, rainfall is the dominant factor, as it varies greatly in intensity and distribution, and generally gives rise to two distinct peaks in the spring and autumn. The study of the behaviour of the house-fly in the tropics should therefore include a study of rainfall.

The behaviour of the house-fly may be considered to be influenced by two sets of factors ; viz., biological, such as feeding, breeding, and resting habits ; and environmental or climatic factors, such as temperature, humidity, rainfall, and light. In each set, one factor may dominate the others, whilst the biological factors as a whole may dominate the environmental, or *vice versa*. For example, in temporary resting places, the feeding and breeding instincts are generally stronger than the environmental, as the familiar presence of flies in kitchens and privies must be due to these instincts rather than to conditions of high temperature and low humidity. In permanent resting places, when the flies are hibernating, feeding and breeding instincts are dormant, and efforts are directed towards retaining body warmth and water balance. A complete quantitative study of each factor is necessary, and all the biological and environmental factors have to be considered when analysing their behaviour under any set of stimuli in the field.

SUMMARY AND CONCLUSIONS.

The study of the common house-fly, *Musca domestica*, L., has not received the attention it merits by medical entomologists. Although the correlation between *fly-borne diseases* and *climatic factors* has interested several workers, this correlation has not been satisfactorily explained. An investigation of the influence of *climatic factors* on house-flies was therefore undertaken.

House-flies can be reared in the laboratory by a proper choice of the breeding medium and suitable technique. Manures, kitchen refuse and synthetic media may be used but the last mentioned is recommended for the production of a supply of standard insects.

M. domestica chooses the lower humidity on each of the humidity gradients, 20–40, 40–60, 60–80 and 80-100 per cent., at a constant temperature of 25°C. The choice is significant for both sexes, and for dry and wet flies, except for dry flies at the range 60–80 per cent.

The house-fly chooses 30°C. in temperature gradients of 20–30°C. and 30–40°C. at constant humidity, expressed either in the R.H. or the S.D. scale. It chooses 30°C. with dry air if possible, but with moist air if it must. In a gradient of 33–27°C. where dry air is associated with 33°C. in the S.D. scale in the one case, and 27°C. in the R.H. in the other, it chooses 33°C. or 27°C. according as it coincides with dry air. Dry air as represented by low R.H. or high S.D. did not make any difference to its choice, consequently it is not possible to decide whether house-flies choose by the R.H. scale or the S.D. scale. The activity of *M. domestica* to different combinations of temperature and humidity shows maximum activity with high temperature ~~of~~ and low humidity, minimum with high temperature and high humidity; while in the case of low temperature combinations with either high or low humidity, activity lies intermediate in degree. High and low temperatures and high and low humidity within themselves also show significant results by the χ^2 test.

The experimental results are explained on physiological grounds and the results obtained in these experiments are compared with those of other workers on similar problems on a variety of insects.

The general experience with regard to house-flies in the field is explained in the light of these laboratory findings.

For a correct analysis of the behaviour of the house-fly in nature, biological stimuli such as feeding, breeding and resting habits must be considered quantitatively in relation to environmental factors such as temperature, humidity, rainfall and light. The present work forms part of such a study.

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References.

AINSWORTH, R. B. (1909). J. R. Army med. Cps., **12,** pp. 485–498.

ALDRIDGE, A. R. (1907). *Ibid.*, **2,** pp. 558–571.

AUSTEN, E. E. (1939). The House Fly.—Econ. Ser. Brit. Mus. (Nat. Hist.), no. 1 (4th edn. revd. by J. Smart), 25 pp.

AWATI, R. R. (1919). Indian J. med. Res., **7,** pp. 549–567.

BACOT, A. W. & MARTIN, C. J. (1924). J. Hyg., **23,** pp. 98–105.

Buxton, P. A. (1931a). Bull. ent. Res., **22,** pp. 431–447.

——. (1931b). Proc. ent. Soc. Lond., (A) **6,** pp. 27–31.

——. (1932). Biol. Rev., **7,** pp. 275–320.

—— & Mellanby, K. (1934). Bull. ent. Res., **25,** pp. 171–175.

Graham-Smith, G. S. (1913). Flies in relation to Disease. Cambridge Univ. Pr.

Gunn, D. L. (1934). Z. vergl. Physiol., **20,** pp. 617–626.

——. (1937). J. exp. Biol., **14,** pp. 178–186.

—— & Cosway, C. A. (1938). *Ibid.*, **15,** pp. 555–563.

—— & Kennedy, J. S. (1936). *Ibid.*, **13,** pp. 450–459.

Hamer, W. H. (1908–10). Reports of the Public Health Committee to the London County Council.

Hewitt, C. G. (1914). The House Fly. Cambridge Univ. Pr.

Howard, L. O. (1912). The House Fly as Disease Carrier. London, J. Murray.

Kennedy, J. S. (1937). J. exp. Biol., **14,** pp. 187–197.

Key, K. H. L. (1936). Bull. ent. Res., **27,** pp. 399–422.

Martin, C. J. (1913). Brit. med. J., 4th Jan., 1913, pp. 1–8.

Mellanby, K. (1933). J. sci. Instrum., **10,** pp. 349–351.

——. (1934). Proc. roy. Soc., (B) **116,** pp. 139–149.

——. (1935). Biol. Rev., **10,** pp. 317–333.

——. (1939). Proc. roy. Soc., (B) **127,** pp. 473–487.

Newstead, R. (1907–09). Report(s) on . . . the common House Fly . . . in Liverpool.

Nicholson, A. J. (1934). Bull. ent. Res., **25,** pp. 85–99.

Niven, J. (1910). Proc. R. Soc. Med., (Epid.), 3 (2), pp. 131–216.

Patton, W. S. & Senior White, R. (1924). Rec. Indian Mus., **26,** pp. 553–577.

Peters, O. H. (1910). J. Hyg., **10,** pp. 602–777.

Richardson, H. H. (1932). Science, **76,** pp. 350–351.

Roberts, E. (1906). Enteric Fever in India. London, Baillière, Tindall & Cox.

Rogers, Sir L. (1928). Indian med. Res. Mem., no. 9, 175 pp.

Russell, A. J. H. & Sundararajan, E. R. (1928). *Ibid.*, no. 12, 204 pp.

Senior White, R. (1934). Bull. ent. Res., **25,** pp. 551–596.

Thomsen, M. (1934). Quart. Bull. Hlth Org. L.o.N., **3,** pp. 304–324.

——. (1938). Stuefluen og Stickfluen.—Beretn. vet.-og Landbohøjsk. landøkon. Forsøgslab., **176,** 352 pp.

—— & Hammer, O. (1936). Bull. ent. Res., **27,** pp. 559–587.

Thomson, R. C. M. (1938). Bull. ent. Res., **29,** pp. 125–140.

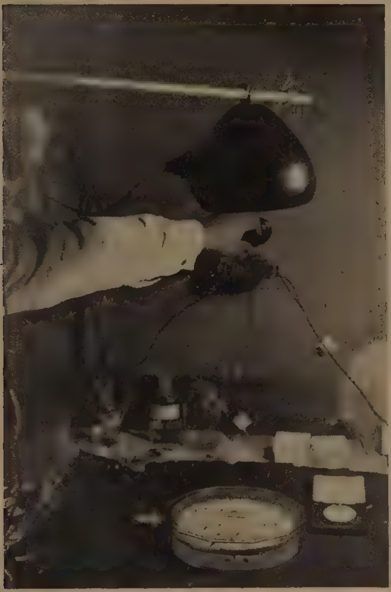

FIG. 1. Humidity Chamber, with the same humidity on both sides, showing scatter of flies by chance and not by choice.

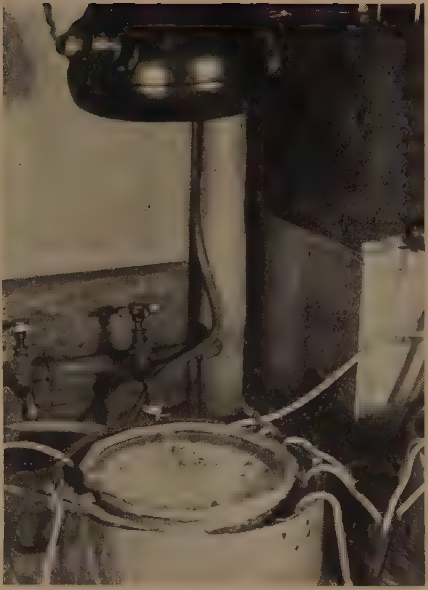

FIG. 3. General view of apparatus for the study of temperature gradients at constant humidity and light. The temperature control is from thermostatic tanks, the light control from a motor headlight above, and humidity control from the floor of the chamber.

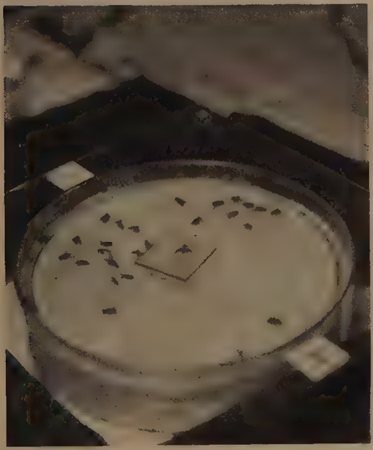

FIG. 2. Humidity Chamber, with humidity gradient 20-40% R.H., showing flies choosing the lower humidity 20% R.H., in preference.

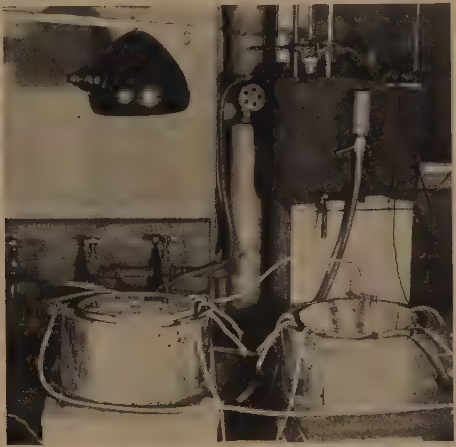

FIG. 4. Temperature gradient apparatus at constant humidity and light. Two parallel experiments with the same gradient of temperature, one with relative humidity constant and another with saturation deficiency constant. (See also Diag. 5.)

THE PENETRATION OF THE INSECT EGG-SHELLS.

I.—PENETRATION OF THE CHORION OF *RHODNIUS PROLIXUS*, STÅL.

By J. W. L. Beament, M.A., Ph.D.
Agricultural Research Council, Unit of Insect Physiology.
Department of Zoology, Cambridge.

Up to the present time there would appear to be very little correlation between the approved routine for the application of ovicidal materials, and the actual mode of entry of such poisons into the egg. O'Kane and Baker (1934 & 1935) investigated the penetration of oils into various insect eggs, by immersing them in liquids containing dyes and sectioning the eggs after treatment. A similar method has been used by Kuenen (1946), who crushed eggs after applying the oil. It is probable that the colouring material in the case of these stained specimens would have penetrated alongside the oil. But both series of experiments are open to the objection that the colouring material could have been filtered out by the shell membranes ; in unstained material the lack of dye is not necessarily an indication that no oil has penetrated. Evidence of this filtering mechanism has been obtained in experiments to be described later. Further, in the experiments of O'Kane and Baker, the eggs were immersed for periods up to 96 hours, which is not at all comparable with the normal conditions of spraying, and may have resulted in oil penetrating through a preferential site of entry, whence it could diffuse through the yolk—even into the shell layers—from the interior.

In a series of recent papers, Beament (1946a, b & c ; & 1947) has described in detail the structure and properties of the shell in eggs of the bug, *Rhodnius prolixus*, Stål. It was pointed out in those papers that the egg of this particular insect was chosen because of its size and availability and because of the mass of information (Wigglesworth, 1942, etc.) on the nymph and adult. The embryology of the egg has also been investigated (Mellanby, 1935 & 1936). Thus, although *Rhodnius* is not a pest of economic importance, these studies provide a " type specimen " for work on eggs of hemipteron pests, and possibly for other orders of insects.

In the present paper an attempt has been made to investigate two problems : the mode of entry of materials penetrating the chorion ; the changes in the subchorial membranes, with their effect on the resistance of the egg. Simple chemicals of comparatively small molecular weight, such as solutions of binary salts, alcohols, fatty acids, etc., have been used rather than the more complex materials recognised as commercial ovicides. This course was adopted because the physical and chemical properties of the simple substances are known. The exact lethal action, once a substance has entered the embryonic organism is not prerequisite to this study. It is intended rather, to indicate the principal differences between the entry of hydrophilic and lipophilic substances, the rôle played by wetting agents, and by such factors as concentration of poison and period of immersion.

The Structure of the Egg-shell.

The egg of *Rhodnius prolixus* (fig. 1A) is of typical homopteran form, elipsoidal, with a maximum length of 2·5 mms. and diameter of 0·7 mms. The unspecialised portion of the shell is sculptured into irregular hexagons, with a follicular pit at the centre of each. A cap at the anterior end is displaced at eclosion, and is joined to the main shell by a pair of thick complex rims.

Beament (1946b) has described the seven layers making up the *chorion*, which he defines as "that part of the extra-oöcytic material which is secreted by the follicle cells of the ovary." These layers may be conveniently divided into four groups (fig. 1B), *viz.*:

(*a*) The *resistant endochorion* (protein with granular material); freely permeable to water soluble molecules of comparatively large size, but impermeable to oils, especially if the layer contains water.

(*b*) The *amber layer* (lipidized protein); impermeable to all but very small particles, and somewhat resistant, even to the passage of water and uniatomic ions.

(*c*) The *soft endochorion* (protein); as (*a*).

(*d*) The *exochorion* (lipoprotein or "chorionin"); despite partial penetration by pore canals, this layer allows only very small particles to pass through it.

A cement substance is scattered irregularly over the outer surface of the shell, and the inner surface is covered by the waterproofing material, or primary wax layer (Beament 1946c).

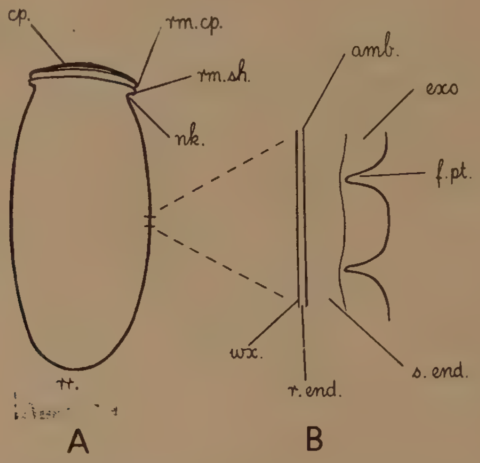

Fig. 1.—The egg of *Rhodnius prolixus*. A. The whole egg. *cp.* cap; *nk.* neck; *rm. cp.* rim of cap; *rm. sh.* rim of shell containing micropylar complex; *rr.* rear end of egg. B. Simplified diagrammatic section through the unspecialised shell of the egg. *amb.* amber layer; *f. pt.* follicular pit; *exo.* exochorion; *r. end.* resistant endochorion protein layer; *s. end.* soft endochorion protein layer; *wx.* primary wax layer on inside of shell.

The cap of the egg is composed of similar layers in order, but units (*a*) and (*c*) are considerably reduced, with corresponding increases in the thickness of (*b*) and (*d*); the total thickness of the chorion is the same.

It is thus apparent at the outset that the greater proportion of the egg's surface is extremely well protected against the entry of most substances, whether hydrophilic or lipophilic. However, between the cap and shell, the *micropylar complex* includes two varieties of capillaries which penetrate some or all of the shell layers. In addition the amber layer is absent around this annulus of the shell, so that the whole complex must be considered in greater detail in relation to the penetration of materials.

The rim of the shell of *Rhodnius* in plan and section is seen in fig. 2 (and see Beament, 1947). The true *micropyles* run from a funnel-shaped orifice in the rim of the shell, through the chorion ; the distal portion of the tube, or *outer micropyle*, has a diameter of approximately two microns and its walls consist of the more lipophilic chorionin. The *inner micropyle*, with a diameter of less than 0·5 micron, is lined with tanned protein. The total length of the tube is approximately twenty microns. The *pseudomicropyles* lie entirely within the chorion. They are about eighteen microns long and two microns in diameter but have a bunch of pore canals leading from the outer end to the surface of the shell. The inner end lies in the resistant endochorion layer. Thus, with the exception of the innermost micron, the tube has a comparatively lipophilic surface.

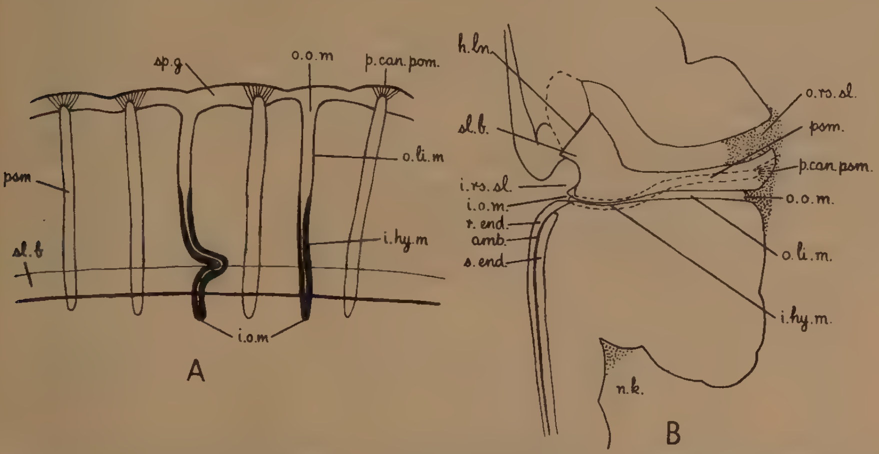

Fig. 2.—The micropylar complex. A. Diagrammatic plan of an arc of the rim of the shell. *i. hy. m.* inner hydrophilic part of micropyle lined with protein ; *i.o.m.* inner opening of micropyle ; *o.li.m.* outer lipophilic part of micropyle lined with lipoprotein ; *o.o.m.* outer opening of micropyle ; *p.can.psm.* pore canals leading to pseudomicropyle ; *psm.*pseudomicropyle ; *sl. b.* sealing bar joining cap to shell ; *sp. g.* spermatic groove. B. Diagrammatic section through rims of shell and cap, passing through a micropyle and showing the relative position of pseudomicropyles. *amb.* amber layer ; *h. ln.* hatching line ; *i. rs. sl.* inner recess of the seal ; *nk.* neck of shell ; *o.rs.sl.* outer recess of seal ; *r. end.* resistant endochorion layers of shell ; *s. end.* soft endochorion layer of shell. (Other abbreviations as in A.) Note : stippled areas indicate position of cement as found on eggs from young females.

The number of micropyles is not constant ; there are approximately fifteen per egg, scattered irregularly around the rim of the shell but the number decreases as the female producing the egg ages (Beament, 1947). There are some 200 pseudomicropyles per egg.

Technique.

Standardisation of materials.

The culture of *Rhodnius* adults was maintained at 25°C. and 60–70 per cent. relative humidity. Each jar of adults contained 20 males and 20 females, together with clean dry blotting paper. Females cement their eggs on to the papers, which were removed each morning and the eggs carefully detached. The eggs were incubated in three arbitrary groups ; those from the females up to eight weeks old, eight weeks to four months, and older than four months. These eggs are designated below as those from young, medium-aged and old females. At 25°C., the eggs take an average of 15·5 days to develop.

The fertility rate and the viability of eggs are most important factors when choosing material for an ovicidal study. In this respect, eggs of *Rhodnius* are outstanding. Over 2,000 eggs from young and medium-aged females were examined in a preliminary study. Less than one per cent. were infertile and at the temperature and humidity used in these experiments, less than one quarter per cent. of the remainder failed to hatch (and see Clarke, 1935). Thus although controls were used in all the experiments recorded below, it can be assumed that the control figure is at least 99 per cent. hatch. Old females lay a rather higher proportion of sterile eggs.

Ovicidal Procedure.

Except where stated in the text, the testing of ovicidal action was carried out in the following way : Eggs were counted into a watch glass and two cubic centimetres of the ovicidal liquid added. Where the ovicide did not wet the surface of the shell, the eggs were immediately submerged, using a fine camel-hair brush ; this was necessary with most aqueous solutions. The only materials used which were more dense than the eggs, were chloroform and carbon tetrachloride. To prevent the evaporation of any volatile components, inverted watch glasses were placed over the preparations. Dipping was carried out at room temperature (approximately 20°C.). At the end of the immersion period, the eggs were removed by a brush, their external surfaces dried rapidly on blotting paper and placed in $\frac{1}{4} \times 1\frac{1}{2}$ inch tubes. These were left on the bench for one hour to allow for the evaporation of volatile materials before incubation.

Immersion in Water.

Eggs were immersed for varying intervals of time in distilled water and in Ringer's solution. Immersion for four to five hours (the longest period used in any of the ovicidal experiments) had no apparent harmful effect on eggs of any stage of development. Fifty to sixty per cent. hatches were obtained from eggs immersed for periods of 24 hours but many of the nymphs which hatched successfully after such treatment, died the following day. It may be that the progressive decrease in survival following immersion in water, or exposure to high humidity, is due to the anaerobic conditions imposed on the embryo.

Criterion of Hatching.

In every ovicidal experiment, eggs must eventually be divided into two groups, those which are killed and those which survive. Many authors (Potter & Tattersfield, 1943 ; Kuenen, 1946, etc.) have referred to the difficulty of making such division, which is necessarily of an arbitrary nature. Indeed it has been shown that some ovicides, such as summer petroleum, do not always prevent development but do inhibit eclosion (Kuenen, 1946). In the case of *Rhodnius* the process of hatching must have started if the cap has been displaced, and after that the organism may make contact with any toxic residues deposited on the outer portion of the shell. As soon as the cap has been displaced death may occur for reasons other than the penetration of a toxic agent through the shell. In the experiments described below, eggs were examined finally after twenty days incubation ; an egg with its cap secure was recorded as dead, and one with the cap displaced, as having survived its treatment. Where development continued for a considerable time after dipping, or where nymphs died shortly after hatching, these facts are recorded.

The Penetration of Materials through the Chorion.

The unspecialised shell and the cap of *Rhodnius* eggs are multi-layered structures ; the nature of the laminae is such that no large molecules will pass through them.

Potash, nitric acid and other corrosive agents may penetrate the outer layers but the under layer is not attacked by them. The following observations are therefore of importance :—

When whole eggs are fixed in Altmann's fluid, the yolk of the egg is slowly blackened due to the fixation of fat by osmic acid. When such eggs were examined after successive intervals of time, it was found that the yolk blackened progressively from the cap end. Similarly, when eggs were placed in glacial acetic acid, cytolysis of the yolk occurred, in the first place, at the cap end. This could be observed through the shell after about five minutes immersion.

Owing to the thick amber layer, the cap of the egg could not possibly transmit materials on the scale demanded by these experiments. It is less permeable than the unspecialised main shell but around the rim of the shell, the amber layer is absent and the micropyles and pseudomicropyles traverse the shell.

In addition to the above experimental evidence, visible changes in the anterior portion of the yolk were observed when eggs were placed in chloroform. Eggs were also immersed in aqueous solutions of water-soluble stains and in oily liquids coloured with red Sudan III. After various intervals of time, they were removed, dried rapidly on blotting paper and placed immediately in liquids with which the stains were immiscible. The micropyle containing rim was dissected from the shell in the liquid and mounted in the same medium. Other of these eggs were cut along their longitudinal axes and mounted in cavity slides so that the cut edges could be observed. More satisfactory evidence was obtained with solutions of basic fuchsin and after immersion in this stain the eggs were transferred to a saturated solution of sodium chloride. This precipitates the dye immediately *in situ* and the optical distortion, unavoidable in the use of immiscible liquids, was thereby obviated. It was found that stains were localised in the true micropyles and occasionally in the pseudomicropyles. The cement residue was sometimes stained, but no part of the exochorion had been penetrated.

The penetration of empty shells.

One-day-old eggs were divided into equal batches and frozen in glycerine jelly. The caps were removed from one group and the rear portions of the unspecialised shell were cut from the others. The yolk was washed out from each egg, using a fine jet of water, and the empty shells were dried in a desiccator. The remaining material consisted of the chorion, with any cement adhering to the outer surface ; on the inner surface (Beament 1946c) there was the primary wax layer and a very permeable fertilisation membrane. The shells were filled with small quantities of powdered stains—stains which were soluble in the particular liquid whose penetration was to be studied. Thin-walled glass capillary tubes were drawn so that they would pass through the neck of the shell, or the openings which had been cut in the posterior ends. Each shell was sealed on to one end of a capillary (fig. 3). Many cements were tested and the most impermeable material was selected for use with each respective liquid. Great care was taken to ensure that, in preparations of the rear part of the shell, the cement covered the whole of the micropylar rim : in preparations of the front end, that the rim was completely uncovered.

Each liquid was placed in a $2'' \times \frac{1}{2}''$ tube and a capillary dipped into it with the shell at the lower end. The upper portion of each tube was held in a split cork. Capillaries were at least two inches long with only the lower half-inch immersed. The preparations were inspected at hourly intervals on the first day and left in position for three days. Any liquid which passed through the chorion, or bypassed it, formed a coloured solution inside the shell, which eventually passed up the capillary. Thus penetration on any reasonable scale was readily observed. At the end of three days, preparations which were apparently impermeable to liquid were dissected to ensure that the stain was still dry and that the capillary was not blocked by cement.

The following liquids were used in the first series of penetration experiments :—

Absolute methyl alcohol
Absolute ethyl alcohol
Absolute propyl alcohol
Absolute butyl alcohol
Acetic acid, glacial
Propionic acid, glacial
Acetone
Benzene
Chloroform
Xylene
Carbon tetrachloride
Petroleum ether

None of these substances could be detected in the shell cavity preparations in which the rear end of the shell was exposed to the liquid for 24 hours. The powdered stain may have been moist in a very few examples. On the other hand all the above liquids entered the yolk space and after varying intervals of time, invaded the capillary when the cap end was immersed. Furthermore, after three days immersion, almost all preparations showed that some of the stain had diffused outwards into the surrounding liquid.

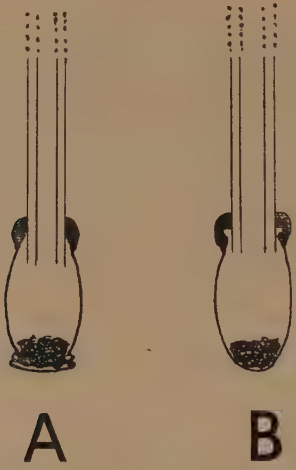

Fig. 3.—Shells cemented to glass capillaries to detect the penetration of liquids. A. Front end of shell with micropylar complex exposed ; B. Rear end of shell exposed, with micropylar complex covered. The empty shells contain powdered dye.

These results can only be explained by assuming that the liquid has traversed the micropyles or pseudomicropyles. Only this portion of the shell is lacking in any of the laminae found in the impermeable rear portion of the shell. In particular, the amber layer is impermeable to the large molecule dyes used in these experiments (Beament 1946b). Penetration through the true micropyles is the only way in which dye could have passed outwards to the liquid surrounding the shell.

An exception to these conclusions occurred when glacial formic acid was used. This liquid passed through rear-end preparations in considerable quantity in the course of 24 hours. Lower concentrations of the acid in water were unable to penetrate (see below). The exceptional behaviour of glacial formic acid must be attributed to its chemical effect on the protein components of the shell.

Any measurement of the actual rate of entry of these liquids is of little interest, since the circumstances of experimental conditions are very different to those existing in the live egg.

In this series of tests all the liquids used have wax-solvent properties. Although the wax layer and the fertilisation membrane have been left on the inside of the shell, these will not prevent the diffusion of such liquids wherever they may penetrate. On the other hand, if water and similar hydrophilic liquids pass through the shell, they will eventually reach the wax layer and be unable to penetrate any further. The micropyles and pseudomicropyles form the most important channels for the access of lipophilic liquids, but a further series of experiments must be undertaken, using hydrophilic liquids.

Experimental.

One-day-old eggs were used as before but after the yolk had been removed, the shells were extracted in boiling chloroform for half-an-hour. This removes the wax layer from the inside of the shell. The empty shells were again mounted on capillaries, using a high melting-point paraffin wax as the cement. The preparations were immersed in the following liquids :—

Water
Ringer's solution
Methyl alcohol 20–80 per cent. in water
Ethyl alcohol 20–80 per cent. in water
Formic acid 20–70 per cent. in water
Acetic acid 20–70 per cent. in water
Propionic acid 20–80 per cent. in water
Acetone 20–80 per cent. in water
2 per cent. solutions of wetting agents in water.

A variety of powdered stains were placed inside the shells to act as indicators of liquid movement. Again, liquids entered the shells whose micropyles were exposed ; later, the surrounding solutions were also coloured. When the rear ends of shells were exposed, the powdered stain remained dry for many hours and at the end of two days there was usually only enough liquid in the shell cavity to bind the dye into a paste.

It is obvious from these experiments that the micropylar region is essential to the transmission of any quantity of liquid. Further investigation (and see below) suggests that small amounts of liquid do not enter the rear-end preparations by direct diffusion. Fluid traverses the exochorion and soft endochorion to the micropylar region and thence circumnavigates the amber layer into the resistant endochorion. Only water and very small particles could penetrate the exochorion to do this.

The general hypothesis of micropylar transmission was subjected to confirmation in the ovicidal experiments set out below. While the above experiments indicate the importance of the micropylar complex in transmitting any *quantity* of liquid, very minute amounts of a toxic material may enter through the main chorion. No thin lamina of any substance (such as the amber layer which is less than 0·2 micron thick over the main shell) is necessarily *impermeable* to a small molecule ; it may have a very low order of conductivity. Ovicidal experiments were designed to overcome this difficulty, as they are a means of detecting the passage of very small quantities of substance.

Ovicidal experiments.

At this stage of the investigation, it would be most expedient to cover the various parts of the shell and to dip these eggs in a toxic liquid. It is, however, almost impossible to apply any suitable material to the front end of *Rhodnius* eggs, without killing them ; even if development should continue after treatment, it may be

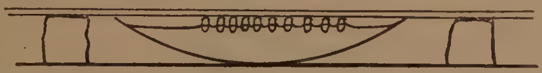

Fig. 4.—Partial dipping in aqueous liquids. Eggs attached by centre of cap to glass rod mounted on plasticine pillars. The rear ends dip in the ovicidal liquid contained in a watch glass.

abnormal. The transition point of the waterproofing wax layer of the egg is 42·5°C. (Beament 1946c); if wax of a higher melting point is applied the egg dies. Most cements are made up in solvents which are toxic to the embryo. Also, a cement over the micropylar region may curtail respiratory exchange.

During preliminary trials it was found that no decrease in toxicity was occasioned when the whole of the unspecialised shell, *excluding* the micropylar area, had been coated in celluloid solution, Canada balsam, etc., and the eggs dipped in a suitable solution. The contribution of any poisons passing through the main part of the shell cannot be very great. Experiments were eventually carried out by dipping only the front half, or rear, of eggs in an ovicidal liquid. This was successful when hydrophilic liquids were used, but lipophiles spread rapidly over the whole shell from any one point. Eggs were attached to a glass rod, using a minute amount of Canada balsam placed either at the extreme rear tip, or in the centre of the cap (fig. 4). This had no toxic effect. Eggs were arranged at intervals of about five millimetres, with their long axes perpendicular to the rod, using rows of ten eggs. The rod was supported at either end on plasticine pillars, with the eggs over a shallow watch-glass.

The ovicidal liquid, coloured with a dye, was carefully poured into the dish, to such a height that it reached half way up the eggs, usually in the form of a meniscus. Thus the liquid could only make its way through the rear part of the shell in one set of experiments and through the front half in the other. After the expiration of the incubation period each shell was examined to discover whether any deposit of stain had been left in the micropyles. The results are shown in Table I.

TABLE I.

Correlation of micropylar entry with kill: front or rear ends of eggs dipped in solution of acetic acid in water with added dye.

Soln.	Part of egg dipped	Time of dip mts.	Age of eggs days	Per cent. kill	Control whole egg dipped	Average number of stained micropyles (m) and pseudomicropyles (psm) per egg			
						Killed eggs		Hatched eggs	
						(m)	(psm)	(m)	(psm)
25%	front	30	1	83	88	12·7	many	0·7	5·3
25%	rear	30	1	14	88	1·0	0	0	0
40%	front	30	1	100	100	many	many	—	—
40%	rear	30	1	30	100	1·7	0·3	0	0
30%	front	30	4	80	77	9·9	many	0	0·2
30%	rear	30	4	9	77	2·0	0	0·03	0
50%	front	30	4	100	100	many	many	—	—
50%	rear	30	4	21	100	2·4	0·5	0	0
30%	front	30	8	78	86	13·5	many	0·1	3·1
30%	rear	30	8	29	86	1·6	1·1	0	0
50%	front	30	8	100	100	many	many	—	—
50%	rear	30	8	15	100	3·1	4·9	0	0
26%	front	30	14	100	98	many	many	—	—
25%	rear	30	14	38	98	1·9	0·25	0	0
40%	front	30	14	100	100	many	many	—	—
40%	rear	30	14	24	100	1·7	3·7	0	0

* Every egg has at least one stained micropyle.

If the micropylar regions of eggs have been submerged in an ovicide, many more eggs are killed than if the rear end only has been immersed. After immersing the front ends, mortalities are equivalent to those obtained in comparable experiments after complete immersion. When the rear ends of eggs are dipped very few eggs are killed ; an examination of those eggs which failed to hatch revealed a deposit of stain in some of the micropyles of every shell. It is indeed certain that a toxic solution need penetrate very few micropyles in order to produce the death of the embryo. In most of these experiments, stain in a micropyle is due to small amounts of liquid which may creep over the surface of the shell. In a few examples, only pseudomicropyles were stained ; the embryos were not killed. No egg survived which had a stained micropyle. Large numbers of one-day-old eggs have remained for half-an-hour with the rear portion in a solution as strong as 50 per cent. acetic acid—they have survived more than ten times the concentration they can tolerate when totally immersed without any mortality. Although the micropyles were less than a millimetre from the surface of these liquids, there was apparently no death due to the acetic acid vapour.

Conclusions.

It is now established that water-soluble materials enter the *Rhodnius* egg by passing along the micropyles. An attempt was made to demonstrate a similar mechanism with oleophilic liquids, but these creep over the shell and the total effect would be the same whatever part of the egg was dipped. However, water enters the micropyles, and this treatment has no harmful effect if eggs are submerged for less than four hours. Eggs were, therefore, placed in water for varying intervals

TABLE II.

The toxicities of coloured oleophilic liquids after eggs have been treated with water and glycerine to occlude the micropyles.

Oleophile	Hydrophile and time immersed mts.	Time in oleo. mts.	Age of eggs days	Percentage kill	Control oil only for same period	Time in oil for percentage kill obtained	Average number of stained micropyles per egg	
							Killed*	Hatched
Chloroform	water							
	10	1	1	35	44	1	2·7	0
	10	2	1	61	72	1·25	5·3	0
	30	1	1	57	44	1·25	9·1	0
	30	2	1	90	72	2·5	7·3	0
	240	1	1	23	44	0·9	3·6	0·03
	240	2	1	52	72	1·25	3·9	0
Benzene	water							
	10	15	4	42	56	13	4·7	0·2
	10	20	4	90	86	21	8·4	0
	30	15	4	58	56	15	many	0·1
	30	20	4	97	86	25	many	0·07
	240	15	4	41	56	13	3·1	0
	240	20	4	50	86	14	4·7	0·25
Chloroform	water							
	10	4	8	16	18	4	3·0	0
	10	6	8	47	59	5·5	6·2	0
	30	4	8	32	18	5·2	many	0·05
	30	6	8	62	59	6	many	0
	240	4	8	4	18	3·5	5·1	0
	240	6	8	18	59	4	4·8	0
Chloroform	glycerine							
	20	1	1	21	44	0·9	2·1	0
	20	2	1	27	72	1	1·6	0
	20	4	8	6	18	3·6	3·4	0
	20	6	8	12	59	4	3·9	0

* Every egg has at least one stained micropyle.

of time, blotted very rapidly on filter paper and transferred to oleophilic liquids. The oils were stained with red Sudan III, so that their subsequent penetration might be followed. The results do not permit such a simple explanation as might have been expected (Table II). Further experiments were carried out using glycerine solutions to block the micropyles against the entry of oil. This liquid was washed out by immersing eggs in water after the experiment; it has the advantage of a high viscosity, so that it is not displaced as rapidly by the oil.

Table II contains the results of all these experiments. Immersion in water may increase or decrease the subsequent toxicity of an oleophilic liquid and this fact is discussed later (p. 377). For present purposes the distribution of stained and unstained micropyles amongst the eggs is important. Eggs which have been killed have a deposit of stain in their micropyles, showing that some of the oleophile has entered them. The surviving eggs may have some deposit of stain in their pseudomicropyles, but their micropyles are not coloured. Particularly interesting is the fact that several of the eggs which were killed had only one stained micropyle; thus, death may be produced by penetration on a very small scale.

These experiments have been carried out with a representative selection of liquids of small molecular size—both hydrophilic and lipophilic in properties. It is very probable that these conclusions also apply to material of larger molecular size, such as the accepted commercial ovicides (except for corrosive substances or those which kill without making contact with the living portion of the embryo).

The Cement.

Even after eggs have been submerged in lipophilic liquids, some of the true micropyles remain unstained. This was noted after considerable periods of immersion and with shells previously dried in a desiccator. The phenomena was most marked in eggs from young females. Again, when corresponding ovicidal experiments are compared, eggs from young females are more resistant than those laid by older ones (fig. 5). Only two differences between these two groups of eggs have been recorded: the number of micropyles per egg-shell (Beament 1947); the amount of cement applied to the shell at oviposition. It is also possible that the over-all resistance of the organism changes—that it depends on the age of the female producing the egg. However, there is no detectable difference in viability between eggs from young and from medium-aged females. Moreover, there are *corresponding decreases* in resistance with eggs at each stage of embryonic development, notwithstanding the magnitude of these changes. This suggests that the factors involved are external to the living organism, rather than within it.

Of the differences in the shell mentioned above, the number of micropyles decrease with the increasing age of the female producing the egg. This would diminish the available paths for transmission of poison and, therefore, the mortalities would be reduced rather than increased. On the other hand, the presence of cement over the outer micropylar orifices might preclude the entry of many liquids and would certainly impede the entry of diffusible fluids. In the ovicidal experiments the most striking changes in toxicity are obtained with oleophilic materials (*e.g.* chloroform, benzene, xylene, etc.). It was concluded that eggs laid by younger females owe their greater resistance to the cement deposited over the outer endings of the micropyles. The properties of the cement were therefore investigated in order to determine its effect on the penetration of various liquids (fig. 2B).

Properties of the Cement.

When the egg of *Rhodnius* is laid, it is secured to a surface by cement. This substance is the secretion of the accessory glands of the female and, in particular, of a large *cement gland*. The cement is a viscous liquid which hardens very rapidly in air on the egg-shell, but more slowly when expressed from an isolated gland.

Young females have greatly distended cement glands ; these were dissected out and the contents expressed on to coverslips. The fresh material, which was a very viscous liquid, was stained quite deeply by basic and acid fuchsin, picric acid and Millon's reagent and gave a ninhydrine reaction. There was no definite polyphenol reaction with ammoniacal silver nitrate solution, although a brownish colour was produced (possibly due to chloride residues, it is also produced in neutral and acid solutions). Proteinaceous material is, therefore, present in considerable quantities. No staining took place in Sudan fat stains in seventy per cent. alcohol.

The cement contained numerous refractile star-shaped crystals ; these stained more deeply in fuchsin and with ninhydrine, than did the matrix. The cement was soluble in cold concentrated potash and in cold strong nitric acid but no oil was liberated during solution ; the crystal-like bodies dissolved more slowly. It is, therefore, very unlikely that the cement contains any lipoproteins. All these properties were also observed with fresh cement layers on the surface of eggs, but whereas prolonged immersion in water does not soften the cement on egg-shells, expressed material was found to swell up after a few hours in cold distilled water. It seems probable that the cement poured out under natural conditions, contains further secretions—probably from associated glands—which " tan " it after it has been secreted. As the egg ages the cement becomes more difficult to stain and more resistant to solution. This supports the view that some slow tanning or polymerisation processes continue after oviposition.

The cement stains intensely in alcoholic solutions of basic fuchsin and this property was used to map its distribution. Whole eggs were immersed in stain and cement deposits were the only *external* portions of the shells which were coloured. Apparently the secretion runs into all the available concavities of the shell ; the eggs of young females have cement in each of the follicular pits of the main shell and of the cap. This closes the pore canals grouped in these outer recesses (Beament 1946b). There is also a considerable deposit in the outer recesses of the seal between the cap and shell (fig. 2B). But it is most important to note that the outer orifices of the micropyles lie in the spermatic groove, whereas the pore canals of the pseudomicropyles open on the convex surface of the rim. Thus cement is often found in the groove where it occludes the micropylar openings ; it rarely covers the pore canals of the pseudomicropyles. In this way the micropyles are protected against the ingress of foreign materials but fluids may enter the pseudomicropyles without restriction. The cement would not, therefore, alter the efficiency of pseudomicropyles acting as respiratory structures (see Beament, 1947 ; Tuft, private communication).

Ovicidal results.

These experiments also indicate that the cement has no water-proofing properties. On the other hand, neither lipophilic nor hydrophilic liquids will traverse it rapidly. Two important observations emerge from the comparison of dosage mortality curves for " young " and " medium " eggs (figs. 5 & 6). The increase in the toxicity of the lipophilic liquids is considerable : hydrophilic liquids are significantly more toxic, but the degree of difference is small. " Medium " eggs are killed by lipophilic liquids after very short periods of immersion. This is due to active wetting of the micropylar capillary (and see p. 376). The period during which a lethal dose passes into the embryo is obviously increased by the time taken for diffusion through the cement plug. Much longer periods of time are usually required for a lethal dose of a hydrophilic liquid to enter the egg. The addition of the time to pass through a cement layer is a relatively small factor, though it is apparent in the results obtained.

Secondly, when " young " eggs are dipped, some eggs are killed by short immersions such as would prove lethal to all eggs in a corresponding " medium " batch. The change in dosage/mortality curves as the female ages is one of decrease in slope and not of movement along the abscissa without change of form. A portion of the eggs in each batch from young females might have cement blocking *all* the micropyles and these eggs would be very resistant. But we have seen above that only one micropyle need be traversed in order that a liquid may kill an egg. Thus other eggs

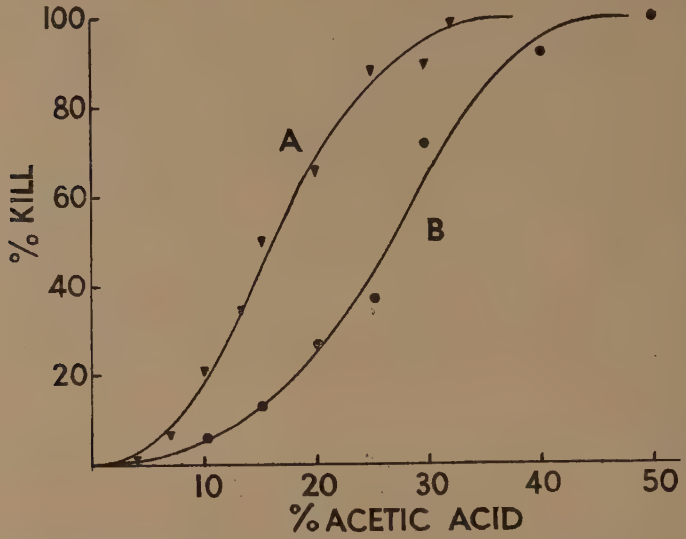

Fig. 5.—Dosage/mortality curves of one-day-old eggs dipped for half an hour in various concentrations of acetic acid in water. A, eggs from medium-aged females. B, eggs from young females.

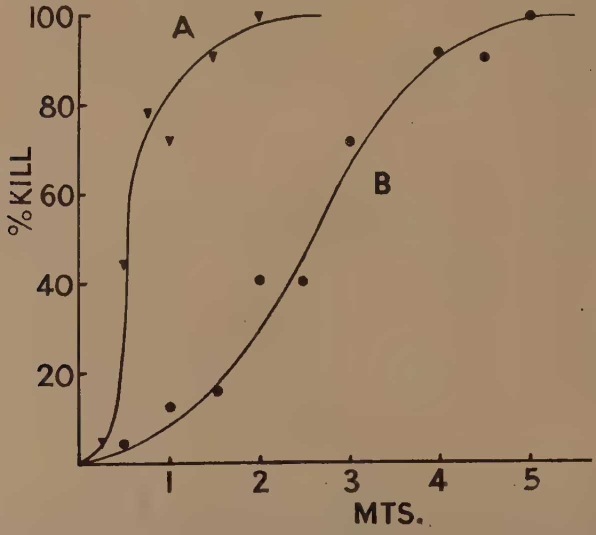

Fig. 6.—Dosage/mortality curves of one-day-old eggs dipped for various intervals of time in chloroform. A, eggs from medium-aged females. B, eggs from young females.

in the batch can be killed more rapidly and some may be as susceptible as "medium" eggs of a similar stage of embryonic development. The uneven distribution of cement on eggs chosen at random, is one factor accounting for deviation between replicates of an ovicidal experiment.

Migration through the Micropyles.

Aqueous liquids.

The outer micropylar capillary is composed of chorionin and this is not readily wetted by water. When aqueous liquids enter a micropyle they present a concave meniscus, tending to retard the progress of the liquid column. Continuous contact with water produces absorption of water molecules on to the capillary surface; consequently the contact angle is reduced and fluid enters more actively. It is for this reason that eggs are always taken from 60 per cent. relative humidity before dipping experiments.

Some experiments were carried out in which eggs from different humidities were subjected to identical exposures in aqueous solutions. There were no very great differences between the kills obtained with eggs taken from 0–90 per cent. relative humidities, but above this level the kills obtained with aqueous liquids were much greater. It is possible that condensation of liquid droplets in the lumen of the micropyle had taken place and this possibility will be considered below.

Imprisoned air.

When a liquid enters the micropylar tube, it encloses a column of air which will be compressed by the advancing interface. This air must, apparently, be dispersed before the liquid can contact the wax layer. Eggs were dipped in aqueous solutions,

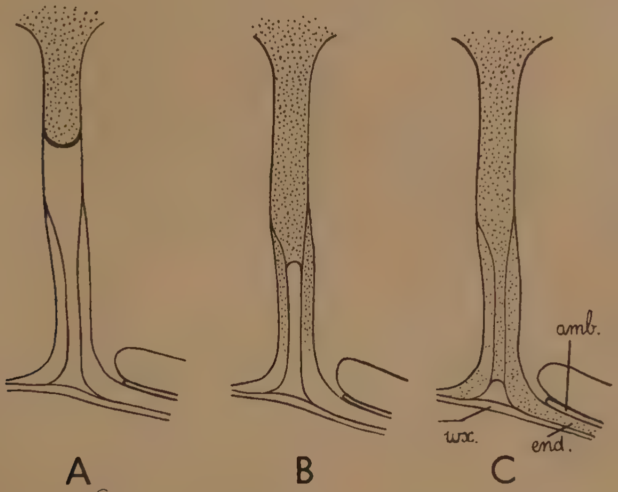

Fig. 7.—Diagrammatic representation of the passage of hydrophilic liquids from a micropyle into the chorion. A, Critical period; liquid moves slowly along outer lipophilic micropyle. B, Absorption period; liquid migrates through protein lining of inner micropyle leaving enclosed air. C, Lethal period; liquid diffusing into inner endochorion layer of shell (*end.*) and approaching wax at base of micropyle (*wx.*) *amb.* amber layer.

such as of acetic acid or mercuric acetate; the period of immersion and the concentration of solute were considerably above the known minimum lethal dose. These eggs were dissected in the ovicidal solution and the micropylar rim mounted in a drop of the fluid, taking great care that it remained immersed throughout the operation. Some of the micropyles contained small quantities of air which filled the inner micropylar ending. Yet these eggs had received a toxic dose of poison *through the wax layer.*

A consideration of the structure of the micropyle (fig. 2) shows that a hydrophilic liquid could be absorbed by the protein lining of the inner micropylar tube, once it has migrated through the outer portion. Providing a liquid, or solute, is of sufficiently small molecular size, it could diffuse through this protein and migrate round the edge of the amber layer into the resistant protein layer of the main shell (fig. 7). The liquid could thus reach the wax layer, having by-passed the air in the base of the micropyle.

Aqueous solutions of large-moleculed stains were used in a first attempt to demonstrate the passage of liquids into the resistant endochorion. They have, however, a slow diffusion rate compared with that of water and do not give any indication of the area through which the water has migrated. The most useful information would be given by the behaviour of small simple molecules, such as those used in the ovicidal experiments. It was found that solutions of potassium permanganate were very suitable. One-day-old eggs were immersed in a 1 per cent. solution and numbers were removed after successive intervals. Groups of eggs with all periods of immersion, between fifteen minutes and six hours, were thus obtained. On removal from the solution, the eggs were dried very rapidly on blotting paper and dropped into absolute

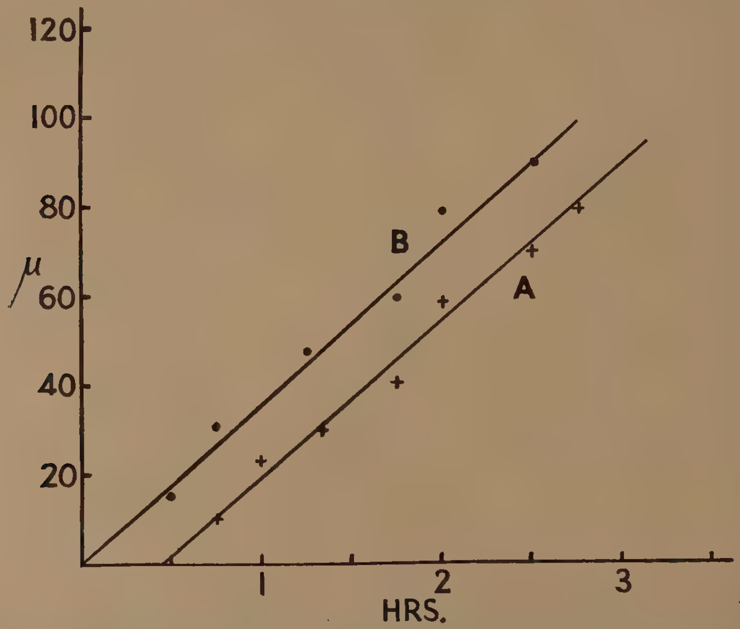

Fig. 8.—Penetration through the endochorion from the micropyles. Graph of the width of the endochorion penetrated by potassium permanganate solution, in increasing time. A, Eggs immersed normally; B, Eggs immersed in evacuated chamber.

methyl alcohol, where they were at once cut into halves along their longitudinal axes. The alcohol invaded the shell from the cut edge and through the inside of the shell, fixing the potassium permanganate instantaneously by reducing it to the black oxide. The halves of these shells were mounted in cavity slides; under the oil immersion objective the exact extent of invasion could be observed and the dark area was mapped by means of *camera lucida* drawings. The experiments showed that the penetrant was limited almost exclusively to the resistant endochorion layer. The potassium permanganate solution passes from the micropyles and forms a dark band round the neck of the shell, which increases in width as time proceeds. The average distance of penetration from the micropyles was plotted against the time of immersion. Confirmatory experiments were carried out, using solutions of mercuric acetate and of silver nitrate; metallic elements were "developed" using hydrogen sulphide and hydrogen chloride respectively. The results were not very satisfactory but provided sufficient evidence for assuming that the mechanism of penetration was not specific to potassium permanganate.

The results of penetration, using potassium permanganate solutions, are shown in fig. 8. The invasion of the endochorion layer is linear with respect to time. Hence the area across which diffusion of poison must take place (the interface between the resistant endochorion layer and the wax) increases linearly. Over any given period of immersion the active surface area will be the integral of this function and is represented by the area beneath the time/distance curve. The probability of death resulting from dipping an egg in an aqueous solution increases as the *square* of time of immersion.

Before relating this principle to the results of ovicidal tests a further conclusion must be considered. It is evident in fig. 8 that the main portion of the curve is linear but that it does *not* pass through the origin. An extrapolation of the curve gives a time value of approximately 30 minutes where it cuts the time axis. Before this period has elapsed no measureable quantity of material has entered the resistant endochorion. This, of course, is the critical period of time during which the column of liquid passes down the lipophilic outer micropyle and through the protein lining of the inner micropyle.

The displacement of imprisoned air is certainly one factor in this slow migration of aqueous liquids along the outer micropyle. Undoubtedly the oxygen component of the air is slowly absorbed by embryonic respiration, accounting for one-fifth of the volume. A small quantity of nitrogen may be dissolved in the advancing liquid but most of the gas will probably be displaced into the surrounding chorion. The critical time interval of 30 minutes may be compared with Wigglesworth's (1931) work on tracheal respiration.

Wetting agents.

Small quantities of detergent materials were added to aqueous tracer solutions. Eggs were dropped on to the surface of the liquids and solutions were chosen which wetted the chorion actively—the eggs sank in them very rapidly. These solutions would present an advancing meniscus in the outer micropylar capillary. Extrapolation of the curve gave a critical time interval very slightly less than the normal (30 mins.). *The slope of the curve was unchanged.* Thus wetting agents do not increase the rate of invasion, once the liquid has reached the protein lining of the micropyle. But the displacement of air is obviously the limiting factor in the process of invasion by aqueous liquids (see p. 380).

The importance of the air-displacement factor was demonstrated in the following way: eggs, enclosed in a gauze cylinder, were placed in a glass vessel (Beament 1946c and fig. 9). Air was removed from the chamber and a tracer solution admitted to the evacuated space; the micropyles thus contained no air to oppose the entry of the liquid column. The solution would, therefore, be forced along the micropyles by the atmospheric pressure when air was again admitted to the apparatus. A time/penetration figure was constructed as before, using these eggs (fig. 8). The curve is linear and parallel to the normal curve but it can be produced backwards *to the origin.* Thus the removal of air enables the liquid to reach the bottom of the inner micropyle almost immediately on immersion, regardless of the high contact angle in the outer capillary.

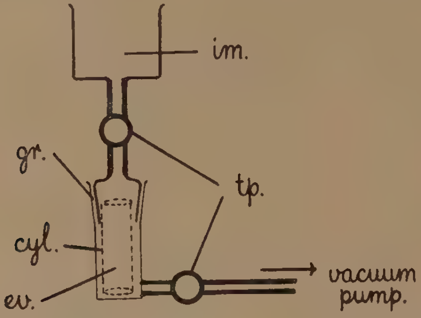

Fig. 9.—Apparatus for immersing eggs under vacuum. Eggs are enclosed in a gauze cylinder (*cyl.*) in the evacuation chamber (*ev.*). *im.* vessel for immersant ; *gr.* ground glass joint ; *tp.* glass tap.

Ovicidal experiments.

In fig. 10 two dipping series are compared : immersion for increasing intervals of time with a fixed concentration of toxic solute, and immersion for a fixed interval

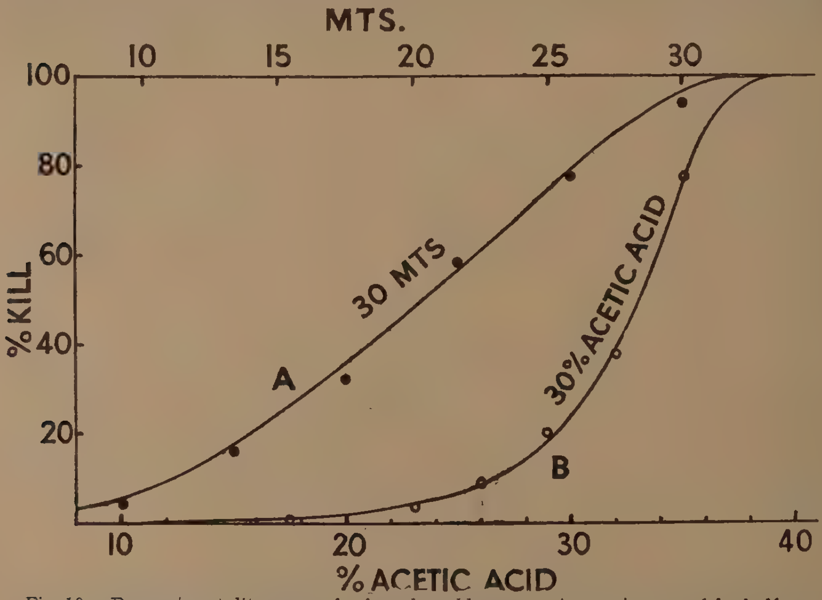

Fig. 10.—Dosage/mortality curves for four-day-old eggs. A, eggs immersed for half an hour using increasing concentrations of acetic acid in water. B, eggs immersed in thirty per cent. acid in water, using increasing intervals of immersion. The curves have been scaled to lie between the origin and a common point of one hundred per cent. mortality.

with increasing concentration of solute. These curves are probably sigmoidal but neglecting the initial and final portions they confirm the views expressed above. With increasing concentration of poison and a fixed interval of immersion, the curve is almost linear. Kill is proportional to an increase in the concentration used, since that much more poison will enter across the area invaded during the fixed immersion time. With increasing time of immersion and fixed concentration of poison, the latter portion of the curve is approximately parabolic, thus, kill is proportional to the integrated area across which the poison diffuses, an area increasing linearly and whose sum is proportional to the square of time. The first portion of the curve represents a time during which almost no kill is recorded ; this is the "critical interval" before the liquid reaches the endochorion protein layer.

TABLE III.

The toxicities of solutions of acetic acid in water after eggs have been submerged in water for various periods of time.

Solution	Time in water mts.	Time in solution mts.	Age of eggs, days	Percentage kill	Equivalent kill for dry eggs in acid for period in acid	Equivalent kill for dry eggs in acid for period (water+acid)
Acetic acid 15 per cent.	10	30	1	81	50	89
	30	30	1	100	50	100
	60	30	1	100	50	100
Acetic acid 20 per cent.	10	30	4	33	32	44
	30	30	4	92	32	100
	60	30	4	100	32	100
Acetic acid 20 per cent.	10	30	8	21	14	29
	30	30	8	55	14	78
	60	30	8	46	14	100

Facilitation of entry.

The factors involved in the entry of hydrophilic liquids are (*a*) the wetting power of the liquid ; (*b*) the displacement of air. Of these factors (*a*) is the less important, it includes the experiments in which the chorion was exposed to various humidities before dipping. In those experiments, saturation of the chorion (which alters the angle of liquid meniscus) did not increase the kills very greatly ; this would be expected. On the other hand, exposure of eggs to saturated air did increase the mortality when the eggs were subsequently dipped. It was suggested then that this may be caused by the condensation of droplets of water in the micropyles. Such an occurrence would be similar to the circumstances in eggs placed *in* water before immersion in an aqueous solution. In the following dipping series, batches of eggs were placed in water for varying intervals and immediately transferred to standard solutions of acetic acid (Table III). The micropyles had filled with water during the initial immersion ; when the egg was transferred to the poisonous solution, solute molecules could diffuse at once to the protein layer and hence diffusion through the wax layer could start almost immediately. The resistant endochorion would not be saturated with water and so it would absorb the poisonous solution in the normal way. For comparative intervals of immersion in the poisonous solution, mortality was increased. The solution at the base of the outer micropyle would be more dilute than the external solution at first, but if the solution was present in excess, the distribution of solute would be equilibrated very rapidly. If, on the other hand, the eggs had remained in water for a much longer period, the endochorion would have an appreciable content of water and would not imbibe the solution actively. It would probably also cause the effective dilution of the solute which could reach the wax layer *only* by diffusion processes. It is obvious that these results have a considerable bearing on the practical application of ovicides. The humidity history of eggs, immediately previous to ovicidal treatment, may be of great importance to the subsequent effect of a water-soluble poison. The water content of the chorion is also a factor which might lead to variation between the replicates of ovicidal experiments, especially in the field.

Before leaving the consideration of hydrophilic liquids, it must be pointed out that the volume of liquid which the micropylar tubes may contain, does not necessarily bear any relationship to the dosage of toxic material which kills the egg. Although it may take several minutes for liquid to reach the protein lining of the micropyle, liquid is, thereafter, absorbed at a considerable rate (see p. 369). Most of the liquid is diffused through the endochorion and does not reach the wax layer at all. Nor must it be assumed that unless eggs are dipped for more than the critical interval, they will survive their treatment. Even if the outer micropyle is not filled with liquid when an egg is removed, respiration and absorption will continue and providing the rate of evaporation of the solvent is not too great, some of the solution may reach the protein of the shell. However, unless the solute is exceptionally toxic, it is unlikely that sufficient will reach the embryo to cause death. Experiments have shown that some eggs may be killed by solutions of mercuric acetate in water after only five minutes immersion ; mercury is exceptionally toxic to insect tissue and the use of mercury salts and vapour against eggs is well known (Rahman & Singh 1946 ; Richards, 1945 ; etc.).

Lipophilic Materials.

Oily liquids also pass down the micropyles to enter the embryonic organism. Compared with aqueous solutions, lipophilic liquids kill an embryo after much shorter periods of immersion (fig. 6). This is not necessarily due to the greater toxicity of the materials under consideration. The majority of the lipophiles used were volatile (*e.g.* chloroform, acetone, absolute alcohol) so that all toxic material would have evaporated from the shell in a short time after its removal from the liquid.

An examination of shells after immersion in, for example, stained solutions of chloroform, shows that liquid has reached the base of the micropyle in a very short time although there may still be bubbles of air in the outer micropyle. Lipophilic materials can wet the surface of dry chorionin very efficiently and, in such circumstances, the liquid has undoubtedly run down between the air and the surface of the outer micropyle. The critical period associated with the outer micropyle is thus very small indeed. Providing the inner micropyle is dry, it will not have a markedly hydrophilic surface, though it will not be as lipophilic as is chorionin. The total air content of the inner micropyle is, however, excessively small and its displacement by respiration, absorption in the shell and solution in the advancing liquid will be a rapid process ; the liquid will be entering under considerable capillarity forces.

A further factor may be involved in the movement of lipophilic liquids in the inner micropyle. It has been shown (Beament, 1945) that the vapour of wax solvents can alter the permeability of organised wax layers. Such might occur as soon as eggs are immersed ; the wax plug at the base of the micropyle might have its permeability increased or it might even be disrupted.

Once lipophilic material in the liquid phase has made contact with the wax layer, the wax will be dissolved and entry will be very rapid (compared with hydrophilic materials), though it will take place through a series of minute apertures. Eggs were immersed for varying periods of time in stained lipophilic liquids. In the majority of experiments, the resistant endochorion was not stained at all, showing that no migration through the protein portion of the shell had taken place. A few liquids, however, did leave a deposit of stain in the endochorion, particularly methyl and ethyl alcohols and to a much less extent, acetone. It would appear that lipophilic liquids which are also miscible with water, are able to enter endochorion protein layers. Such liquids are less efficient wax solvents and this restricts their activity later (see p. 380).

Effect of Humidity and Water.

The surface of the micropyle becomes more hydrophilic as it absorbs water. Dipping experiments with lipophilic liquids were carried out after eggs had been exposed to various humidities. Using such active lipophilic liquids as, for example, chloroform, it was not possible to demonstrate a significant effect due to humidity ; only small numbers of eggs were used. It is possible, that, just as chloroform can pass along the interface between air and the micropylar surface, it can also do so, displacing the adsorbed water-molecules. The entry of benzene seems to be more delayed.

Where eggs were *immersed* in water before dipping in an oil, the resultant mortality was considerably affected (p. 368). An analysis of the results obtained produces unexpected conclusions. If eggs are immersed in water for a time less than its critical interval (30 mins.), the lethal period of immersion for the oleophile may be somewhat extended. Following this the lethal period is not extended and may even be reduced. Finally, when eggs have been in water for a long time, the lethal period required by the lipophile is much increased (Table II).

After a short immersion in water, the liquid will not have reached the inner (protein) micropyle. The lipophile, therefore, encounters a column of water when it enters the outer micropyle. This liquid may be forced down the inner micropyle (whence it would be absorbed, leaving the inner micropyle wet, but free for migration to the wax) ; or water may be by-passed, once the oil has made contact with a chorionin surface, just as a bubble of air is sometimes left.

Once the water column has reached the inner micropyle, active absorption takes place. The volume of the outer micropyle is approximately ten cubic microns and, from fig. 8, the endochorion is invaded by water at a rate of 200 cubic microns per minute. Assuming that fifteen micropyles are participating in the uptake of water, each micropyle will transmit liquid at the rate of 200/15=13 cubic microns per minute. Hence all the water in one micropyle will be replaced every 10/13=0·7 minutes. Therefore, supposing the outer micropyles are filled with water the liquid will only be a barrier to the entry of an immiscible lipophile for some forty seconds. Moreover, its movement ensures that the lipophile does enter the micropyle. The addition of this interval will not make a significant difference to the period in which an egg receives a lethal dose of a lipophilic liquid. This accounts for the considerable difference between blocking the micropyles with water and with glycerine ; the latter is not absorbed by the endochorion and remains an effective barrier (see Table II).

When, however, the egg has been in water for a considerable time, the resistant endochorion will be partially saturated and its rate of imbibing water will be diminished. A proportionally longer period of time must then be added to the penetration period of the oil (Table II). If the inner layers of the egg become so saturated that the inner micropyle is temporarily filled with water, then it will be virtually impossible for a water-immiscible oil to reach the wax layer and the egg will be completely protected. We have found, however, that if *Rhodnius* eggs are immersed for such a length of time, death will ensue without the necessity for using an oleophilic liquid. Nevertheless, the implication of these results on the treatment of eggs in the field may be very considerable, especially if a high concentration of water in the shell of a particular species does not affect the development of the embryo (see p. 382).

The Wax Layer.

It has been indicated above (Beament 1946a, c) that the *Rhodnius* egg is protected throughout its life by a wax layer on the inner surface of the endochorion. This envelope surrounds the embryo *completely*, and is the principal mechanism resisting both desiccation and the entry of water-soluble toxic materials. The layer of wax, probably about 0·25 micron thick, covers the inner openings of the micropyles and is produced before fertilisation. It is penetrated by the fertilising spermatazoon but is repaired within a few hours of fertilisation. During this very short period of time, eggs may be susceptible to water soluble poisons but it was not possible to obtain sufficient numbers of these eggs, so soon after oviposition, to carry out dipping tests.

Under normal circumstances, therefore, hydrophilic substances must pass through the wax and other properties being equal, the transport of a water soluble substance having but slight oil affinity will be facilitated (see Webb & Green 1945). It would thus be advantageous for an ovicide (acting on a *Rhodnius* egg) to have both oil and water solubility. Wax solvents penetrate very rapidly through minute holes at the bases of the micropyles. The degree of disruption of the wax layer is not sufficient to produce death by desiccation. One-day-old eggs (Table II) require approximately three minutes immersion in chloroform to receive a lethal dose ; the majority of eggs are killed by two minute dips. But five minutes immersion does not produce the collapse of eggs, even if they are transferred to a desiccator for twenty-four hours instead of incubating them at 60 per cent. relative humidity. Older eggs which require five minutes immersion for 100 per cent. kills, do not collapse completely when desiccated after an hour in chloroform. The wax solvent materials are evidently killing eggs by direct entry and by direct toxic action at some site in the embryo. The majority of these liquids are so volatile that there can be no question of death resulting from a film of the wax solvent altering the permeability of the shell.

However, wax solvents have some unusual effect on the embryo. If eggs are dipped in these liquids for intervals giving approximately 50 per cent. kills the surviving *nymphs* die very shortly after emergence and it would appear from their bodies, that death was due to desiccation. This phenomenon was noted after using benzene, chloroform, carbon tetrachloride and 100 per cent. acetone. Eggs were, therefore, incubated in atmospheres of high humidity after immersion, in the hope that this would decrease waterloss. It merely had the effect of reducing the number of successful eclosions (Clarke, 1935).

The solvent may remain dissolved in the wax layer for some considerable time after dipping, but it is very unlikely that the production of the first nymphal wax layer would be affected.

Penetration of the wax layer.

It has been suggested that materials which kill an egg in a very short period of immersion do so because of wax-solvent action, or solubility in the wax. These two factors are very different. They are clearly separated in ovicidal experiments using various concentrations of alcohols and ketones in water. It is known (Beament, 1945, 1946c) that absolute ethyl alcohol is an efficient, though not vigorous, solvent of insect waxes ; 90 per cent. alcohol in water is by no means as efficient. On the other hand these two liquids have almost identical wetting properties when applied to chorionin and to tanned protein. There will be very little difference between the rates at which the respective fluids flow to the bottom of the micropyles. The solubility of alcohol in the wax layer will be unaltered by the presence of water, but the toxicities of the two liquids to the one-day-old eggs are very different (fig. 11).

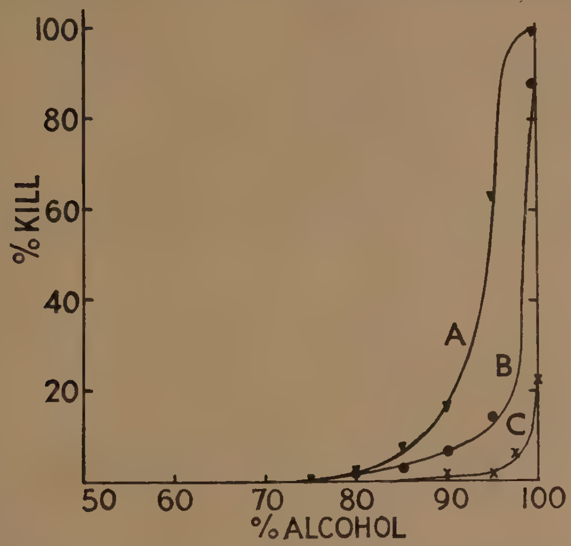

Fig. 11.—Dosage/mortality curves of one-day-old eggs dipped in various concentrations of alcohols for half an hour. A, eggs from medium-aged females dipped in ethyl alcohol; B, eggs from young females dipped in ethyl alcohol; C, eggs from medium-aged females dipped in methyl alcohol. Note steep increase in mortality where the liquid has wax solvent properties.

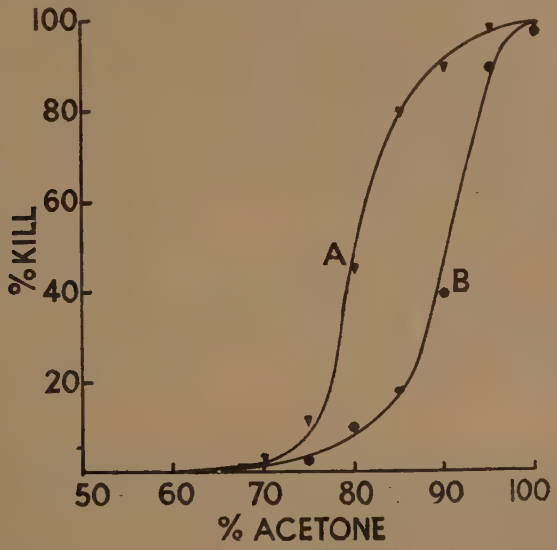

Fig. 12.—Dosage/mortality curves of one-day-old eggs dipped in various concentrations of acetone in water for half an hour. A, eggs from medium-aged females. B, eggs from young females.

Increasing the concentration of alcohol from 30 to 90 per cent. does not alter the subsequent mortalities appreciably, but the change from 90 to 100 per cent. causes an enormous increase in toxicity. This sudden change is apparent with eggs at all stages of development ; the primary wax layer is present throughout. The volume of ethyl alcohol which enters an egg dipped in a 90 per cent. solution will depend on the rate of diffusion of the alcohol through the wax. When absolute alcohol is used, the wax dissolves and the embryo can imbibe alcohol freely.

The dosage/mortality curves (fig. 12) for solutions of acetone in water confirm the action of ethyl alcohol. The ability of acetone to dissolve wax decreases very markedly when small quantities of water are added. Further, and supporting evidence, was obtained by using methyl alcohol. Compared with ethyl alcohol, absolute methyl alcohol is a very poor wax solvent and though methyl alcohol is usually very much more toxic to living material than is its higher homologue, fewer eggs are killed in equivalent periods of immersion (fig. 11).

Emulsifier and Detergents.

Since wax solvents enter *Rhodnius* eggs very rapidly, aqueous ovicides should be made more efficient if they contain substances capable of disrupting a wax layer. Some of the effects of detergents and wax emulsifiers were next investigated. Wigglesworth (1945) has demonstrated the increased permeability of insect cuticle, when such materials are applied to the integument. Some emulsifiers have been used (p. 373) to decrease the contact angle in the outer micropyle and thus decrease the critical interval of an aqueous solution. The corresponding ovicidal experiments were not then discussed, since it was realised that wetting agents might alter other factors, particularly the penetration of wax layers. The materials were selected from those employed in the studies of Wigglesworth (1945) and Beament (1945). One per cent. of each emulsifier was added to solutions of 4 per cent. acetic acid in water. When applied for a period of half an hour, 4 per cent. acetic acid is not toxic to eggs at any stage of development ; deaths following the use of a solution containing an emulsifier will, therefore, be due to the facilitated entry of acetic acid (less a control figure for the toxicity of the emulsifier alone).

TABLE IV.

The toxicity of solutions of 1 per cent. of various wetting agents and emulsifiers applied to one-day-old eggs for 30 minutes and when combined with 4 per cent. acetic acid. In one series air has been removed from the micropyles by vacuum immersion. Four per cent. acetic acid alone is not toxic for 30 minutes immersion and it is possible that oily materials *may* kill eggs merely by blocking the respiratory channels.

Emulsifier and type	CO9993 hydro	R2211 oily	G.L.S. hydro	Prol. hydro	D.G.O. hydro	C.H.S. oily	R2206 oily	GE1117 oily
Percentage kill with 1 per cent. emulsifier only ...	36	25	16	9	0	10	54	13
Wetting of emulsifier on egg-shell	Med.	Med.	Med.	Good	Poor	Poor	Med.	Poor
Percentage kill, 4 per cent. acetic acid + 1 per cent. emulsifier ...	63	59	39	51	77	36	60	27
Wetting of emulsifier + acid ...	Part ppte	Good	Poor	Ppte	Ppte	Poor	Med.	Poor
Percentage kill with vacuum immersion, acid + emulsifier	71	91	97	57	82	33	79	22

The results of these dipping experiments (Table IV) were disappointing, especially the mortalities resulting from solutions containing R.2211 and C.09993; both these substances are known to be very efficient emulsifiers of insect cuticular waxes. Further investigation was made using the vacuum immersion apparatus (p. 374) and by extending the period of immersion to one hour. After either treatment the percentage kill was increased. The micropylar rims of eggs dipped in the normal way were examined; a considerable concentration of emulsifier material was found in the junction of the inner and outer micropyle and alongside the distal portion of the inner micropyle. This was particularly visible in eggs dipped in the rather yellow R.2206.

It has been shown on p. 371 that when eggs are placed in aqueous solutions, the liquid is first absorbed at the junction of the inner and outer micropyles, leaving air in the inner tube. A liquid containing emulsifier does not behave any differently and for some time after immersion, absorption takes place at the surface of the protein-lined capillary. But large detergent molecules, often arranged in larger stable miscelles, will not enter the protein. They accumulate at the entrance of the inner micropyle and until respiration and displacement have continued for some time, the emulsifier does not make contact with the wax. Mortality is not increased, because the acetic acid is diffusing to the embryo in the same way as it would if the emulsifier were not present.

After vacuum immersion, however, the emulsifier reaches the wax layer at once and the acid can pass through—mortalities in these experiments are higher (Table IV). Similarly, after an hour's immersion the liquid will have reached the bottom of the inner micropyle (fig. 7). Whether the emulsifier is present or not, a small moleculed toxic material will still diffuse into the endochorion in the normal way and so the increased mortality given by solutions containing emulsifiers is entirely due to the action of the emulsifier itself. When an emulsifier is applied to an insect cuticle, the amount of water permeating depends on the extent of disruption in the wax layer and the permeability of the film of emulsifier through which water is lost (Beament 1945). In the present experiments, only the increased permeability of the wax layer is important. Hence, for example, R.2211 is more efficient in increasing the entry of acetic acid than is C.09993. On insect cuticle, C.09993 causes most evaporation of water. But a layer of R.2211 is comparatively impermeable to water, though it is probably the more efficient wax emulsifier. (Some emulsifiers are affected by the addition of 4 per cent. acetic acid; direct comparisons of materials may not be justified.)

Control figures.—One per cent. solutions of some of the emulsifiers in water alone are toxic to eggs. Some of these eggs were transferred to a desiccator for 24 hours after immersion, to determine whether waterloss was responsible for death. No egg collapsed more rapidly than a normal egg or one killed at a corresponding stage with cyanide. The results are similar to those obtained with wax solvents (see above). Even when the emulsifier makes contact with the wax layer it is doubtful if the area and degree of wax disorganisation is at all great. It is not surprising that some of the solutions of these emulsifiers are toxic. Their effect on cell walls and other components of the embryo may be considerable. D'Arcy Hart (in a private communication) states that C.09993, at a concentration of 1/100,000 in water is lytic to red blood cells.

Discussion.

The majority of ovicidal experiments have been performed on one-day-old eggs. The uniformity of this material permits the comparison of results and also, these eggs are more easily killed than are eggs of any other stage of development owing to the lack of extra-embryonic membranes. It must be pointed out that the ovicidal experiments displayed above to illustrate the other work are representatives of ovicidal tests with a wider range of liquids ; it is not intended that this paper should contain any indication of materials suitable for killing eggs and the ovicidal experiments should be treated as illustrations.

In dipping tests, the volume of liquid which has been used to immerse eggs has been far in excess of the volume of the individual egg. A very small amount of this liquid enters the shell and it is this volume which contains the lethal dose. If the shell already contains water, redistribution of a solute between the internal liquid and the solution will not lead to an appreciable drop in concentration. But if eggs are sprayed, the total volume of liquid on the external surface of the shell may be small and only that liquid which is contiguous with the micropylar openings, or perhaps, with the spermatic groove, will invade the endochorion. Water in the endochorion may cause a very considerable dilution of the solute and this may be of particular importance if it alters the wax-solvent properties, or wax-affinity, of the invading liquid (p. 380). If eggs are sprayed, the greater portion of an aqueous liquid may be left on the shell surface. Thus, wetting agents may increase the volume of spray-droplets which are confluent with the micropylar openings, for the droplets will run together (and see David, 1946).

Summary.

The unspecialised portion of the shell and the cap of *Rhodnius* eggs are impermeable to almost all hydrophilic and lipophilic liquids. If water and very small ions pass through the chorion they must traverse a wax layer on the inside of the shell. Certain corrosive materials, *e.g.*, glacial formic acid, may pass through the shell slowly.

These conclusions, based on experiments with pieces of shell, have been confirmed in ovicidal experiments. A range of materials with widely differing properties enter the embryo only through the micropyles, of which there are approximately fifteen in the rim of each shell. At least one micropyle must be traversed to kill an egg but many eggs were killed when only one had been penetrated.

A cement, applied by the female at oviposition, may occlude the outer orifice of a micropyle. The properties of the cement are described ; it appears to be a tanned protein. Cement deposits are much more copious on the eggs laid by younger females. Such eggs are more resistant to ovicides because penetration is delayed. This increased resistance is more pronounced when oleophilic liquids are used owing to the rapidity with which they kill eggs from older females. The random distribution of cement is one cause of the variability of replicates in ovicidal tests.

A detailed investigation has been made of factors governing liquids traversing the micropyles. Hydrophilic liquids invade the outer lipophilic part of the micropyle slowly ; the displacement of air is the most important factor and small changes in the wetting power of the liquid make little difference to the rate of entry.

Aqueous liquids are absorbed into the protein lining of the inner portion of the micropyle. They reach the wax layer on the inside of the shell by migrating into and through the inner protein layer. The area which is invaded increases linearly with time. Mortality, therefore, increases as the square of the time of immersion, but it is proportional to the increase in concentration of a solute if the period of immersion is constant.

Oleophilic liquids wet the micropyle actively. They may by-pass air and flow rapidly to the wax at the inner end of the tube. Wax solvents kill very quickly and are much more toxic than other lipophiles.

Water in the micropyle and shell may affect the entrance of either type of liquid. In general it increases the toxicity of aqueous solutions and retards the entry of oils.

Wax-emulsifying materials added to aqueous solutions do not produce great increases in toxicity. They are " filtered out " at the protein lining of the micropyle and do not reach the wax layer for a considerable period of time.

Acknowledgements.

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References.

BEAMENT, J. W. L. (1945). The cuticular lipoids of insects.—J. exp. Biol., **21,** p. 115.

——. (1946a). The waterproofing mechanism of an insect egg.—Nature, Lond., **157,** p. 370.

——. (1946b). The formation and structure of the chorion of the egg in an Hemipteron, *Rhodnius prolixus*.—Quart. J. micr. Sci., **87,** p. 393.

——. (1946c). The waterproofing process in eggs of *Rhodnius prolixus* Stähl.—Proc. roy. Soc., (B) **133,** p. 407.

——. (1947). The formation and structure of the micropylar complex in the egg-shell of *Rhodnius prolixus* Stähl.—J. exp. Biol., **23,** p. 213.

CLARKE, N. (1935). The effect of temperature and humidity upon the eggs of the bug, *Rhodnius prolixus*.—J. Anim. Ecol., **4,** p. 82.

DAVID, W. A. L. (1946). The quantity and distribution of spray collected by insects flying through insecticidal mists.—Ann. appl. Biol., **33,** p. 133.

KUENEN, D. J. (1946). Het winterei van het fruitspint (*Metatetranychus ulmi* Koch) en zijn bestrijding.—Tijdschr. PlZiekt., **52,** p. 69.

MELLANBY, H. (1935). The early embryonic development of *Rhodnius prolixus*.—Quart. J. micr., Sci., **78,** p. 71.

——. (1936). The later embryology of *Rhodnius prolixus*.—Quart. J. micr. Sci., **79,** p. 1.

O'KANE, W. C. & BAKER, W. C. (1934). Studies of contact insecticides VIII. A technique for tracing penetration of petroleum oil in insect eggs.—Tech. Bull. N.H. agric. Exp. Sta., no. 60.

—— & ——. (1935). Studies of contact insecticides IX. Further determination of oil penetration into insect eggs.—Tech. Bull. N.H. agric. Exp. Sta., no. 62.

POTTER, C. & TATTERSFIELD, F. (1943). The ovicidal properties of certain insecticides of plant origin (nicotine, pyrethrins, derris products).—Bull. ent. Res., **34,** p. 225.

RAHMAN, K. A. & SINGH, G. (1946). Mercury as a preventative against stored grain pests.—Bull. ent. Res., **37,** p. 131.

RICHARDS, O. W. (1945). The effects of mercury vapour on the eggs of *Calandra granaria* L.—Bull. ent. Res., **36,** p. 283.

WEBB, J. E. & GREEN, R. A. (1945). On the penetration of insecticides through the insect cuticle.—J. exp. Biol., **22,** p. 8.

WIGGLESWORTH, V. B. (1931). The extent of air in the tracheoles of some terrestrial insects.—Proc. roy. Soc., (B) **109,** p. 354.

——. (1942). Principles of Insect Physiology. London, Methuen.

——. (1945). Transpiration through the cuticle of insects.—J. exp. Biol., **21,** p. 97.

A SIMPLE METHOD FOR BREEDING THE HOUSE-FLY, *MUSCA DOMESTICA*, L., IN THE LABORATORY.

By M. Hafez, M.Sc., Ph.D., F.R.E.S.

Department of Entomology, Faculty of Science, Fouad I University, Cairo.

Various substances have been used by different workers as substrata for breeding the house-fly under laboratory conditions. Horse manure was recommended by Hutchison (1916), Glaser (1924), Grady (1928), Derbeneva (1935) and Kusina (1936). A mixture of horse manure and hog manure was used by Hockenyos (1931). Feldman-Muhsam (1944) obtained the best breeding results by using cow dung. Pig dung was preferred by Lörincz & Makara (1935). Lodge (1918) reared house-flies on a mixture of casein, bread, water and banana. Richardson (1932) used another mixture of wheat bran, alfalfa meal, diamalt, yeast and water. Kobayashi (1935) used a by-product of soya beans. Basden (1947) in his large scale experiments, necessitating about 2,500 flies daily, used a mixture of middlings, grass meal, tap water, dry malt extract and dried yeast.

About 100 fly larvae were required daily for experimental purposes at the Zoological Laboratory, Cambridge. The following method for rearing the larvae of *Musca domestica*, L., proved to be the simplest and most satisfactory.

A petri dish (9 cm. in diameter and 2·5 cm. in height) containing a piece of cotton wool moderately soaked in diluted milk (3 volumes milk added to 1 volume water) was placed in the breeding cage with the adult flies. These were fed on the milk and the females laid their eggs on the milk pad. The latter was replaced by a fresh pad every 24 hours. After the eggs had been laid, the pad was transferred to a two-pound jam jar containing a fresh milk pad to provide food for the hatching larvae. The jar was tightly covered with a finely perforated tin lid and kept in the incubator at 27°C. Fully grown healthy larvae were obtained after four or five days. Prior to pupation, the larvae usually collected at the upper and drier surface of the milk pad which by this time became blown up into a more or less spongy mass due to the continual tunnelling of the larvae inside and probably to the onset of fermentation. At this upper surface, the larvae usually pupated. Pupae were either collected or preferably left in the jar to complete development. The emerging adult flies were then transferred to the breeding cage to maintain the stock.

Table I shows that the duration of development is shorter in the case of the method described than when Richardson's medium is used.

TABLE I.

Breeding medium	All experiments at 27°C.		Total duration of development from egg to imago in days
	Duration of larval stage in days	Duration of pupal stage in days	
Richardson's	6–8	4–6	11–15
Milk	5–6	4–5	10–12

The above method may be recommended for rearing house-fly larvae in moderate numbers (100–200 larvae daily) on the grounds of its simplicity. The relatively laborious preparation of Richardson's or other similar artificial media is avoided and natural media such as horse manure or pig dung, etc., are not always easily obtainable under laboratory conditions. Furthermore, in order to make conditions more favourable for oviposition, these media—natural or artificial—have to be prepared in fresh quantities every day. In addition the larvae obtained by this method are quite healthy and normal and are easily collected from the breeding jar.

The best breeding results were obtained when the following precautions were taken:

Moderate soaking of the cotton wool pad in diluted milk. Excessive soaking may cause the drowning of the ovipositing females in the pad, and may also check egg or larval development.

Avoidance of excessive numbers of developing eggs in the milk pad to prevent the crowding of the hatching larvae.

Careful tightening of the lid otherwise larvae may escape from the breeding jar.

The above work was carried out at the Zoological Laboratory, University of Cambridge, and my thanks are due to Captain J. L. Cloudsley-Thompson for checking the proofs.

References.

Basden, E. B. (1947). Breeding of the house fly (*Musca domestica*) in the Laboratory.—Bull. ent Res., **37,** p. 381.

Derbeneva, P. V. (1935). On the number of generations of *Musca domestica.*—Med. Parasitol., Moscow, **4,** p. 404.

Feldman-Muhsam, B. (1944). Studies on the ecology of the Levant house fly (*Musca domestica vicini* Macq.).—Bull. ent. Res., **35,** p. 53.

Glaser, R. W. (1924). Rearing flies for experimental purposes, with biological notes.—J. econ. Ent., **17,** p. 486.

Grady, A. G. (1928). Studies in breeding insects throughout the year for insecticide tests. 1. Houseflies (*Musca domestica*).—J. econ. Ent., **21,** p. 598.

Hockenyos, G. L. (1931). Rearing house flies for testing contact insecticides.—J. econ. Ent., **24,** p. 717.

Hutchison, R. H. (1916). Notes on the preoviposition period of the house fly, *Musca domestica* L.—Bull. U.S. Dep. Agric., no. 345.

Kobayashi, H. (1935). The influence of temperature upon the development of larvae of *Musca domestica.*—Trans. Dynam. Develop., **10,** p. 385.

Kusina, O. S. (1936). Fecundity and preadult mortality in *Musca domestica* L.—Med. Parasitol., Moscow, **5,** p. 329.

Lodge, O. C. (1918). An examination of the sense reactions of flies.—Bull. ent. Res., **9,** p. 141.

Lörincz, F. & Makara, G. (1935). Observations and experiments on fly control and the biology of the house fly.—L. O. N. Hlth Org., C. H. Hyg. rur. E. H.5.

Richardson, H. H. (1932). An efficient medium for rearing house flies throughout the year.—Science, **76,** p. 350.

STIMULI INVOLVED IN THE ATTRACTION OF *AËDES AEGYPTI*, L., TO MAN.*

By Alec H. Parker, M.Sc.
Department of Zoology, University of Glasgow.

The recent expansion in research on mosquito repellents has resulted in a revival of interest in the means whereby mosquitos locate their hosts. Largely because of its amenability to laboratory manipulation, *Aëdes aegypti*, L., has figured prominently in these investigations. In the experiments to be described in this paper, some attempt has been made to assess the rôle of some of the stimuli associated with the human body, in guiding females of this species to man. Stimuli which have received attention are smell, warmth, and moisture. Visual stimuli have not been investigated.

Probably the most detailed study on the behaviour of *A. aegypti* to date has been that of Kennedy (1939). Kennedy's interest centred primarily on visual responses, but the study of these led him to draw certain inferences regarding host location. He observed, for example, that females would follow a black stripe moving over a white background, and concluded that the reaction might be an important means of attraction to the moving host. In investigating orientation in a current of air, he found emanations from the human body to have a strongly activating effect. Flying females normally travelled upwind, but they took off very much more readily when the wind carried human emanations than when no such stimuli were present. The result of these reactions was that they flew towards the source of stimulation.

The "emanations" to which reference is made comprise those emitted in the form of breath, and those arising from the surface of the body. In both cases warmth, moisture, and smell, are component stimuli. Among those who have made some attempt to investigate the relative effects of such stimuli is Reuter (1936). Reuter's work has been obtainable only in abstract, but he is reported to have tested the reactions of *A. aegypti* females to various constituent acids of sweat, and to have found these substances "unattractive". Moisture proved "attractive", and warmth "essential for biting". Reuter's negative results with sweat would appear to be borne out by Gordon's (1922b) failure to discover any preference on the part of female *Aëdes* for individuals differing in degree of sweating. As Gordon himself points out, however, the number of observations made was small, and the significance of the result is questionable. Reuter's alleged claim that warmth is essential for biting is contradicted by sundry observations (Gordon, 1922a; Shannon, 1931; Woke, 1937) testifying to the readiness with which *A. aegypti* females feed on the cold dead bodies of warm-blooded hosts, and on the living bodies of poikilotherms. That warmth is at least of some importance, however, is suggested by Woke's (1937) observation that though *Aëdes* females did not refuse cold blood, they fed very much more readily when the preparation was warmed.

In the above discussion of Reuter's results, the word "attractive" is enclosed in inverted commas. This is because it is not clear whether what is described is that which incites the mosquito to go towards it, to alight on it, or to probe. The present investigation is concerned almost exclusively with the first two types of reaction, and the word attractive will be used only in connection with these.

Since the experiments described below were concluded, I have had access to two reports dealing with recent investigations on mosquito repellents. Both contain accounts of experiments relevant to the subject of this paper, and they will be discussed in a later section.

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* The experimental work described in this paper was done in the Department of Zoology, University of Birmingham.

Material and Methods.

The breeding technique employed was essentially similar to that recommended for *Aëdes aegypti* by David, Bracey, and Harvey (1945), and no further comment need be made here. Only those females which were to be used for maintenance of stocks were given a blood feed; those to be subjected to experimental tests were allowed to feed only on sugar solution. In view of the desirability of obtaining as rigorous a standardisation of experimental conditions as possible it would also have been advantageous to use females of only one specific age group. Unfortunately, the limited number of adults available made this impossible; the most that could be done was to record the maximum possible age limits of the females used in each experiment.

All experiments were carried out in a room maintained at a temperature of 28±1°C., and a relative humidity of 50–70 per cent.

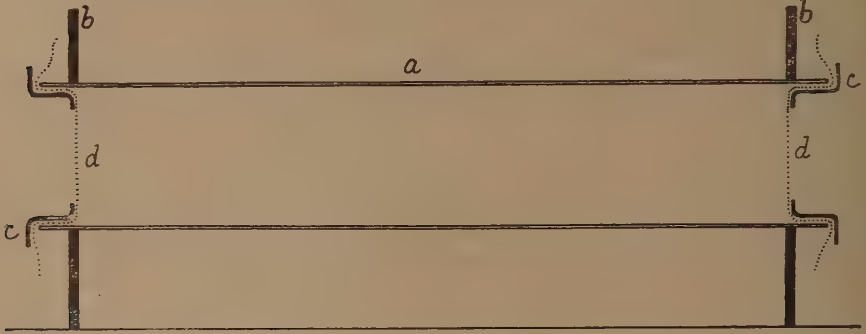

Fig. 1.

The apparatus used is shown diagrammatically in fig. 1. It consisted of a wide glass tube *a*, 29 cm. in length and 5 cm. in diameter, supported at each end by being tightly fitted into a hole in each of two screens, *bb*. A metal cup *c*, fitted into each end of the tube. The base of each metal cup was pierced by a large hole, and a piece of netting *d*,* was stretched over each of the latter. When, therefore, the palm of the experimenter's hand (for example) was pressed against the end of the tube, it was possible for its warmth, smell, and moisture, to diffuse through the netting and into the interior of the tube. The central part of the tube was fairly brightly lit by diffused overhead illumination. The inner side of each screen was covered with white paper, and the whole apparatus placed inside a similarly lined chamber. The only gaps in the latter were spaces in one wall for observation of the apparatus and for insertion of the experimenter's hands.

Female *Aëdes* were collected from the rearing cages by means of a large sucking tube. They were then lightly anaesthetised with ether vapour, and in this condition placed inside the glass tube. Thirty was selected as being a convenient number of insects on which to make observations of the type described below, and this number was strictly adhered to in all experiments. After an interval allowing for full recovery from the anaesthetic, the number of females on one of the netting partitions was counted at fixed intervals (usually of $\frac{1}{2}$ minute) over a given period (usually 5 minutes). The object to be tested was then placed against the same end of the tube, and the number of females on the netting again counted at equal intervals over the same period. The whole procedure was repeated at least twice. It is important to notice that the mosquitos could not actually touch the test object, either with their feet, or with their probosces. They were separated from it by a distance equal to the depth of the metal cup, *i.e.*, approximately 1 cm.

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* The material used was "Scrim"; this is similar to muslin, with the important difference that the interstices are considerably larger in proportion to the diameter of the threads.

For convenience in comparing the results of different experiments, the counts made of the number of females on a given netting partition in the absence of the test object ("control"), and in its presence ("test"), have been combined into a single figure. This figure, which will be referred to as Index of Attraction (I_a), is the test count expressed as a percentage of the combined test and control counts. That is :

$$I_a=\frac{100.\mathrm{T}}{\mathrm{T}+\mathrm{C}}\ T \text{ being the test, and } C \text{ the control count.}$$

From this, it follows that an I_a of 100 indicates maximum positive response, of 50 indifference (because in this case $T=C$), and of 0 maximum negative response.

Response to Human Body Surface.

It appeared that the first thing to establish was the response which female *Aëdes* would exhibit to the sum total of emanations from the body surface. To determine this the palm of the hand was placed against the end of the tube and treated as the test object. Table I, which gives the results of a series of such tests, shows that the number of females on the netting partition was consistently larger when the hand was present, than when it was not.

TABLE I.

Response to untreated body surface.

Age in days	No. of counts made	T	C	I_a
6–19	25	245	162	60·2
1–10	25	254	167	65·7
5–21	40	390	288	57·5
2–6	40	315	174	64·4
1–5	20	144	98	59·5
2–6	40	373	198	65·3
2–5	40	476	202	70·2

T=Total number of females on the netting in presence of hand.
C= ,, ,, ,, ,, absence ,,
I_a=Index of Attraction.

This difference might have been due, either to more females having alighted on the netting in response to the stimuli emitted by the hand, or to the fact that while the number landing remained unchanged, the presence of the hand produced a greater tendency to remain on the partition. In view of this, reference may be made to a series of counts which were subsequently made of the numbers of females leaving the netting (*a*), in the presence of, and (*b*), in the absence of, the hand. The totalled results of these were as follows :—

Number leaving when hand present=503
,, ,, ,, absent = 13

This highly significant difference points clearly to the conclusion that the observed differences between the values for T and C given in Table I were, in fact, the result of more females having landed on the netting when an area of body surface was in the vicinity than when no such object was present.

Response to stimuli emitted by the hand was further demonstrated by the vigorous probing movements made by many of the females alighting on the netting at the baited end ; such movements were never observed during control periods.

Response to a Source of Warmth.

A suitable source of warmth was obtained by allowing warm water to flow through a horizontally placed conical flask ; the flat bottom of the latter formed the warm surface to be tested. The warm water flowed from a large tank, the temperature of which could be maintained at any specified level by means of a thermostatic control. The level at which this thermostat had to be set, in order that the temperature of the warm surface would be equal to that of the human body surface, was determined as follows. The thermostat was set at a known level, and the warm junction of a thermocouple placed, firstly, in contact with, and secondly, at various distances from, the warm surface under consideration. Meanwhile, a set of readings was taken from the mirror galvanometer included in the thermocouple circuit. The procedure was then repeated with the thermostat set at various different levels. Next, the warm junction of the thermocouple was placed in the same positions in relation to the palm of the hand as it had been in relation to the warm surface, and a second series of readings taken. Finally, by comparison of the first series of readings with the second, the level at which the thermostat in the tank had to be set in order that the warm surface would be at the same temperature as the hand, could be inferred. The result was found to be the same for all positions occupied by the thermocouple in relation to the surface tested. (Actually, of course, body surface

TABLE II.

Response to a source of warmth.

Age in days	Temperature of warm surface	Hand			Warmth		
		T	*C*	*Ia*	*T*	*C*	*Ia*
1–6	*b*°C.	102	50	66·7	74	70	51·4
6–10	*b*°C.	174	70	71·3	105	99	51·0
0–24	*b*+1°C.*	101	40	71·4	65	66	50·0
0–6	*b*+1°C.*	288	160	61·7	207	252	45·1
1–7	*b*+1°C.*	170	94	64·4	86	80	51·8
20–30	*b*+1°C.	133	80	62·4	96	104	48·0
4–14	*b*+1°C.	131	44	74·9	50	53	48·5
5–15	*b*+2°C.	145	92	61·2	48	85	36·1
2–19	*b*+3°C.	170	56	75·2	58	69	45·7
3–20	*b*+4°C.	123	43	74·1	78	59	56·9

T = Total number of females on the netting in presence of the test object.
C = ,, ,, ,, ,, absence ,, ,,
I a = Index of attraction.
b °C. = Body surface temperature.

* Experiments in which the warm surface was blackened.

temperature varies slightly at different times, and the mean of several readings obtained with the palm of the hand had to be adopted.) The above procedure having been completed, the thermostat was set, either so that the warm surface was at the surface temperature of the palm (*b*°C.), or so that it was a known number of degrees above this level.

Response to the warm surface was tested in exactly the same way as that described for the hand in the previous section. In addition, as a check on the condition of the mosquitos, the response to the palm of the hand was again evaluated in each experiment ; the two types of stimulus were tested alternately.

Table II gives the results. They give no indication of the warm surface having exerted any effect—attractive or repulsive—either at, or above, the temperature of the body surface. Experiments in which the amount of heat radiated was increased by blackening of the warm surface with lamp black gave equally negative results. Another indication of the indifference of the females was the absence of any probing movements ; the presence of the hand, it will be remembered, produced marked effects of this nature.

Notwithstanding the apparent lack of response to the warm surface, it is conceivable, on the evidence so far presented, that the latter induced a high degree of activity, and that while the number of females *on* the netting at any one time was no greater when the warm surface was in the vicinity than when no test object was present, many more did in fact alight, shortly to fly away again. If this were true the activating effect of the warmth would have been masking its true attractive effect. It seemed highly improbable that it was so, since the activity of the females appeared to be no greater in one case than in the other. However, the possibility was tested by counts of the number of females alighting on the partition. These were made during the experiments just described, a procedure which would have been impossible if the females had been more active. The totalled results for the whole series were as follows :—

Number alighting in presence of warm surface=69
,, ,, absence of any test object=66

Since the correspondence between these two figures, and also between the numbers counted *on* the netting at given intervals is so close, we may conclude that no marked increase in activity was induced by proximity to the warm surface, and that, under the conditions described, a warm surface exerts no attractive effect on female *Aëdes*.

Relative Response to Sweat, Moisture, and Warmth plus Moisture.

Response to moisture was tested by the use of a small filter paper (diameter, 5 cm.) soaked in water at room temperature. As with the other test objects, this was placed against the end of the glass tube. The combination of warmth with moisture was achieved by placing a similar filter paper moistened with warm water against the warm surface described above. By this arrangement the temperature of the water was maintained at body surface level. Sweat (and probably a certain amount of sebum) was collected by holding a filter paper under the experimenter's armpit long enough for it to become thoroughly soaked ; in practice, 5 minutes was usually found to be sufficient. It was tested one minute after collection, by which time there was no question of its temperature being above that of the room.

The effect of all these three stimuli was to produce a congregation of females on the netting in their vicinity, and it seemed that it might be possible to obtain some information about their relative importance as attractants. Accordingly, a series of experiments was carried out, in each of which the hand, sweat, moisture, and warmth plus moisture, were tested in succession. As before, each test exposure was preceded by a control.

Table III gives the results of twelve such experiments. If, now, the values for T and C shown in this table are totalled, and an Index of Attraction for each of the four types of stimulus calculated on this basis, the following figures are obtained :—

Ia for hand =69·6 per cent.
,, sweat =73·3 ,,
,, moisture =69·1 ,,
,, warmth plus moisture=65·5 ,,

These results, assuming for the moment that the differences between them are significant, are surprising. The hand appears to be less attractive than cold sweat, and the attractive effect of moisture to be reduced when it is warmed. Before such conclusions are accepted, however, the activating effect of the various stimuli must be taken into account.

TABLE III.

Response to hand, sweat, moisture and warmth + moisture.

Age in days	Hand			Sweat			Moisture			Warmth+Moisture		
	T	C	Ia	T	C	Ia	T	C	Ia	T	C	Ia
0–7	76	48	61·3	88	40	68·7	105	56	65·2	93	66	58·5
0–7	69	40	63·3	132	41	76·3	130	49	72·6	93	63	59·6
1–8	141	67	67·7	168	20	89·4	179	55	76·5	117	65	64·3
1–9	54	20	72·9	40	22	64·5	57	33	63·3	65	34	65·7
9–14	110	49	69·2	156	55	73·9	146	62	72·4	133	66	66·8
10–15	163	76	68·2	130	39	76·9	139	55	71·6	144	76	65·5
11–16	107	44	72·6	167	66	71·7	122	76	61·6	133	72	64·9
5–12	132	48	73·3	153	88	63·5	150	75	66·7	128	33	79·5
3–8	90	42	68·2	113	39	74·3	79	33	70·5	75	60	55·6
1–9	60	18	70·5	51	27	56·7	65	33	66·3	76	44	63·3
2–5	100	47	68·0	127	46	69·4	111	35	76·0	111	37	75·0
2–6	57	22	72·2	41	15	73·3	57	36	61·3	39	20	66·1

T=Total number of females on the netting in the presence of the test object.
C= ,, ,, ,, ,, ,, absence ,, ,,
Ia=Index of attraction.

During the above experiments, it was very noticeable that the degree of activity induced varied with different stimuli. In the presence of the hand, and of warmth plus moisture, females probed through the interstices of the netting with great vigour, and actively moved to and fro over its surface. In doing so they continually disturbed each other. Since the reaction to being disturbed was almost invariably to fly away, large numbers were continually taking off from the partition and landing again. With the other two stimuli—moisture and sweat—these effects were much less marked.

It was clear that if the attractive power of the four stimuli described was to be compared, these activating effects had to be taken into account. The fact that periodic counts of the numbers on the partition yielded a lower figure for one stimulus than for another, might have been due solely to a difference in activating effect ; the greater the degree of activation induced by a stimulus, the higher the proportion of mosquitos attracted towards it which would have been hovering in the air in its vicinity, rather than remaining on the netting itself.

To assess the influence of these activating effects, the number of females leaving an end partition was counted for given periods in the presence of each of the four types of stimulus. Conditions were exactly the same as those prevailing in the previous series of experiments.

TABLE IV.

Number of females leaving the netting partition in the presence of various stimuli.

Age in days	Hand	Sweat	Moisture	Warmth+ Moisture	Blank
3–8	92	52	52	116	6
5–12	113	33	32	128	0
11–16	98	51	30	103	5
10–15	200	73	59	229	2
Totals ...	503	209	173	576	13

Table IV gives the results of four such series of tests, and shows (*a*), that the number of females leaving the netting in the presence of any of the four types of stimulus was greater than the corresponding number when none of these stimuli were present, and (*b*) that the number leaving the netting in the vicinity of the hand, and of warmth plus moisture, was very significantly higher than the corresponding number for cold moisture and sweat.

It was stated earlier that the extent to which a tendency to take off from the netting was exhibited appeared to be related to the degree of probing activity. This was borne out by observations on the proportion of females on the netting which were probing in the presence of the various stimuli. Such observations, made during some of the experiments quoted in Table III, yielded the following average results :—

Per cent. probing in the presence of the hand =52·9
" " " " warmth plus moisture =59·3
" " " " moisture =21·9
" " " " sweat =20·3

These figures correspond sufficiently closely to those given in Table IV to be highly suggestive of some connection between the activities on which the two sets of readings are based.

Returning, now, to the bearing of these differences in activity on the results given in Table III, we may note the following points :—

The Hand and Sweat : The hand evidently had a much greater activating effect than sweat, and to this the higher Index of Attraction obtained for the latter may reasonably be attributed.

Moisture and Warmth plus Moisture : The lowering of the Index of Attraction when warmth was added to moisture may also be explained by the greater degree of activation induced as a result of this addition.

Sweat and Moisture : Here the differences are less obvious and it becomes necessary to apply significance tests. Table IV shows the number of females leaving the netting to have been greater for sweat than for moisture, and the significance of this difference has been tested as follows. The Standard Error of Proportion, δp, was calculated from the formula $\delta p = \sqrt{\frac{\Sigma a \; \Sigma b}{n}}$, where a and b are the two sets of values under consideration, and n the number of observations. In the present case n was $\Sigma\, a + \Sigma\, b$. Student's t was then determined by estimating the difference of $\Sigma\, a$ or $\Sigma\, b$ from their mean, and dividing this figure by δp. By this procedure, t was found to be equal to 1·8. Reference to the appropriate tables showed this to correspond to a probability of ·065 (65 chances in 1,000) of the difference having been due to chance. Its significance must therefore be regarded as questionable.

The significance of the difference between the Indices of Attraction for sweat and moisture has likewise been estimated. In this case the procedure was rather different, as the figures to be compared were themselves proportions, based on the combined results of two sets of figures. Consequently, δp was worked out separately for each set of Indices. The formula used for this was $\delta p = \sqrt{\frac{p\,(1-p)}{n}}$, where n is the number of observations and p the proportion. In the case in question n was the total number of females observed (*i.e.*, $\Sigma\, T + \Sigma\, C$), and p the Index of Attraction. This having been done for both sweat and moisture, t was found from the formula $t = \frac{M_1 - M_2}{\delta m_1 - m_2}$, where M_1 and M_2 are the means of the respective proportions (in the present case, the Indices of Attraction to moisture and sweat), and $\delta m_1 - m_2$ the Standard Error of their difference. If δp_1 and δp_2 were the two Standard Errors of proportion obtained previously, $\delta m_1 - m_2$ was $\sqrt{\delta p_1^{\,2} + \delta p_2^{\,2}}$.

From these calculations, t was found to be 3·9 ; this means that the probability of the difference having been due to chance is considerably less than 1 in 1,000. In other words, the number of females counted on the netting when sweat was present, was very significantly higher than the corresponding number when moisture was the test object. Since the difference between the numbers leaving the netting in the presence of the two stimuli was hardly significant, and the figure was in any case higher for sweat than for moisture, it seems reasonable to conclude that the sweat actually did exert a greater attraction than the moisture.

Hand and Warmth plus Moisture : One would expect to obtain further information concerning the rôle of olfactory stimuli from a comparison of the response to these two sources of stimulation. It is clear at the outset that the attraction exerted by the warmth-moisture combination did, at least, approach that of the intact body surface ; unlike any of the other constituent stimuli tested, it yielded both an Index of Attraction, and a figure for the number leaving the netting, which were on a level comparable with the corresponding figures obtained with the hand. The possibility of small differences remains, however, and statistical analysis is again required. The mean Index of Attraction for the hand was 69·6 per cent. For warmth plus moisture the corresponding figure was 65·5 per cent. Applying significance tests in the same way as before, a probability of 1 in 1,000 of this difference having been due to chance is obtained. The number leaving the partition was greater with warmth plus moisture than with the hand, the probability of this difference being attributable to chance being 1 in 50. The Index of Attraction for the hand was thus significantly higher than that for warmth plus moisture ; the difference between the numbers leaving the partition doubtfully so. It is therefore impossible to draw any definite conclusion ; the dubious difference between the latter may or may not account for the difference between the former.

Discussion.

In the experiments described, female *Aëdes* appeared indifferent to a source of warmth. That the latter did, at least, have an appreciable effect under some conditions, however, is evident from a comparison of the figures obtained with warm and cold moisture. While both these were found to be attractive, their activating effects were markedly dissimilar. There is just a possibility that this discrepancy was due to differences in the degree of saturation produced in the two cases, but it is much more likely that it resulted from a more direct effect of the difference in temperature. As regards the Indices of Attraction, the mean value for cold moisture (69·1 per cent.) was higher than that for warm (65·5 per cent.). This difference was undoubtedly partly, and probably wholly, due to the greater activating effect of the latter, and the apparent disparity in the degree of attraction exerted is not to be taken at its face value.

The fact that the warm surface exerted a perceptible effect only when it was associated with moisture suggests that a certain degree of humidity may be necessary for a reaction to warmth to be induced. If this is true, the drop in R.H. which must automatically have occurred in the vicinity of the warm surface in those cases in which it was not accompanied by moisture, may be of considerable significance. Further reference to the bearing of differences in humidity on response to warmth will be made shortly.

Sweat, as has already been pointed out, appeared to be slightly more attractive than moisture alone. This, presumably, was due to the operation of olfactory stimuli. The fact that their effect was not very marked suggests that under the conditions of the experiments, at least, their rôle in attraction is not a very important one. This conclusion is fortified by the consideration that warm moisture apparently exerted very nearly, if not quite, the same effect as the untreated body surface. It is as well to remember, however, that the warmth plus moisture arrangement may have produced an unduly high local humidity, so presenting this latter stimulus in more concentrated form than did the palm of the hand.

Similar results regarding the attraction exerted by temperature and humidity have recently been circulated in a report issued by a team working on mosquito repellents in the U.S.A. (DeLong *et al.*, 1945). These investigators found that when a stream of warm air was directed on to the organdie-covered side of a cage containing *Aëdes aegypti*, no attraction resulted. If, on the other hand, the air was moistened, the attraction of the mosquito to the side of the cage became " equal to, if not greater than, that of the human hand held on the opposite side of the cage". Similarly, while a warm plate placed against the side of the cage proved to possess " some attractive value ", the insertion of a moistened filter paper between the cage and the plate greatly enhanced this. In the latter case, it appears that the insects were actually able to touch the source of stimulation, so that results are not strictly comparable with those so far discussed. Granted this difference, however, DeLong's observations are fully consistent with the results arrived at in the present investigation.

DeLong and his collaborators also observed the reaction of *Aëdes* females, subjected to various conditions of temperature and humidity, to an area of human body surface. When offered the latter in an atmosphere of which the humidity was at saturation point and the temperature at 34°C., no response occurred. This, they claim, suggests that the only important stimuli involved in attraction are temperature and humidity. Their inference is that the lack of response was due to the elimination of the temperature and humidity gradient which normally guided females to the body surface. Such an interpretation is open to question. While it may be that the suggestion is correct, the result can equally well be explained on the grounds that the abnormally high temperature and humidity of the experiment exerted a strong conditioning effect, which might have altered the reactions of the insect to stimuli of any description. In the absence of information as to which, if either, of these explanations is correct, no valid conclusions concerning the mechanism of attraction can be drawn.

Experiments on stimuli attractive to *Aëdes aegypti* females have also been carried out at Cambridge, by Sir Rickard Christophers (Christophers, 1947). As in the experiments described in the present paper, mosquitos were confined in a wide glass tube closed at each end by a netting partition, and their reactions to stimuli applied at one end noted. In most of Christopher's experiments, the source of stimulation was 3–4 mm. from the netting. This was somewhat nearer than was the case in the experiments presented here ; in these, it will be remembered, the stimulating surface was approximately 1 cm. from the netting. In neither case, however, could the females actually touch the test object.

Christophers demarcated the tube into two equal lengths, so that the number of mosquitos in each half could be counted. The attraction towards a given stimulus applied at one end was then assessed as follows : at given intervals the number of females on the netting at the stimulated end was added to the number located in the same half of the tube, the figures so obtained being compared with the corresponding total for the other side. The latter was not subject to any particular stimulation. By this technique, Christophers found a warm plate of temperature about 40°C. to be distinctly attractive. The addition of moisture increased this effect, but not to any very marked extent. Moisture at room temperature had " no attractive effect ".

At first sight, these results appear to be largely in contradiction to those given earlier. To some extent, at least, this may be due to differences in the temperature and humidity at which the experiments were done. The experiments described in the present paper were done at 28°C. and an R.H. of 50–70 per cent. Those of DeLong and others were done at the same temperature, and almost the same humidity (40–70 per cent. R.H.). The experiments of Christophers, on the other hand, were done at 25°C. and 95 per cent. R.H. It is quite possible that both these differences are important. The response to warmth may be more marked at 25°C. than at 28°C., and if, as was conjectured earlier, a response to warmth is exhibited only when the humidity is above a certain value, the difference in R.H. may also be of significance. Inequalities in R.H. might also have been responsible for the contradictory results of experiments on the response to moisture at room temperature.

Another point which may be of consequence is that the mosquitos used by Christophers were allowed to feed only on water prior to an experiment. In DeLong's experiments, and in those presented here, some form of sugar solution was available during this period. It is possible that this affected the reaction to a source of warmth, but if this is so, it is strange that the same sugar-fed insects responded so readily to certain other stimuli.

The divergent results obtained by different investigators emphasises the need for caution in applying to a wider field conclusions drawn from experiments done under one particular set of conditions. Another consideration to be borne in mind in evaluating the results of experiments on the host-finding process is that the movement towards, alighting on, and biting of the host by a mosquito, may be a very complex series of reactions. Perhaps different stimuli operate in the initiation of each of these three stages of the response. Such differences, if they exist, remain largely undetected by experiments of the type described in this paper. No distinction has been made, for instance, between the reactions of going towards, and of landing in the neighbourhood of the source of stimulation. The observed congregation on the netting partition certainly involved a landing reaction, but the extent to which the movement towards the bait preceding this was *directed*, rather than merely random, was not investigated. The biting reaction, again, poses further questions which it has not been within our scope to attempt to answer. Only further experimental analysis can elucidate these problems.

Summary.

The reactions of *Aëdes aegypti* females to certain stimuli normally associated with the human surface body have been investigated. The apparatus used was such that the insects were unable to touch the source of stimulation. Attraction to the latter was indicated by the congregation of females in its vicinity.

The following stimuli proved attractive: the intact palm of the hand; collected sweat at room temperature; moisture at room temperature; moisture at body-surface temperature. A warm surface at body-surface temperature produced no apparent effect, either of attraction or of repulsion.

The four types of stimulus found to be attractive also had marked activating effects. The magnitude of these effects was much greater for the hand and warm moisture, than for cold sweat and cold moisture.

Due allowance for the complication introduced by these differences in activation having been made, cold sweat appeared to be slightly, but significantly, more attractive than cold moisture. A reaction to olfactory stimuli is presumed to have been responsible for this. Such stimuli, however, are not considered to have been of much importance in comparison with warmth and moisture; moisture at body-surface temperature had very nearly, and possibly the same, attractive effect as the intact hand.

The results are compared with those of other recent investigations, and the need for caution in applying them where conditions differ from those under which they were obtained is emphasised.

Acknowledgements.

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References.

CHRISTOPHERS, S. R. (1947). J. Hyg., **45**, pp. 176–231.

DAVID, W. A. L., BRACEY, P. & HARVEY, A. (1945). Bull. ent. Res., **35**, pp. 227–230.

DELONG, D. M. *et al.* (1945). Rep. nat. Res. Coun. Insect Contr. Comm., no. 176. Washington.

GORDON, R. M. (1922a). Ann. trop. Med. Parasit., **16**, pp. 229–234.

——. (1922b). *Ibid.*, **16**, pp. 425–439.

KENNEDY, J. S. (1939). Proc. zool. Soc. Lond., (A) **109**, pp. 221–242.

REUTER, J. (1936). Oriënteerend onderzoek naar de oorzaak van het gedrag van *Anopheles maculipennis* Meigen bij de voedselkeuze.—Proefschr. Rijksuniv. Leiden, 118 pp. (R.A.E., (B) **24**, pp. 223–225).

SHANNON, R. C. (1931).—Proc. ent. Soc. Wash., **33**, pp. 125–164.

WOKE, P. A. (1937).—J. Parasit., **23**, pp. 310–311.

THE *LEUCOPTERA* LEAF MINERS OF COFFEE ON KILIMANJARO.
I.—*LEUCOPTERA COFFEELLA*, GUÉR.

By F. B. Notley, M.Sc., A.I.C.T.A., F.R.E.S.
Coffee Research Station,
Agricultural Department, Tanganyika Territory.

Introduction.

Coffee Leaf Miner, identified as *Leucoptera coffeella*, Guér., has been recorded from the West Indies, Central and South America, Central Africa, Madagascar, Reunion and Ceylon (Ferrière, 1936). In East Africa it is found wherever coffee is grown. *Leucoptera caffeina* (Washbourn, 1940), which was described as a new species, previously erroneously identified as *L. daricella*, Meyr., from Moshi, is present on coffee in the Northern and Tanga provinces of Tanganyika, but apart from this its distribution is in some doubt. Specimens in the British Museum from Umkomaas, one from Natal, 1914, and one from Kenya, 1922, this latter from coffee, were identified as *L. daricella* by Meyrick, but are believed by Washbourn (1940) not to be that species ; possibly they may be *L. caffeina*. The late Mr. Ritchie sent specimens from *Cremnospora africana* from Moshi which Washbourn believes are not conspecific with *L. caffeina*. From the latter, Wilkinson (1936) described the parasite *Mirax leucopterae*, which has not been found attacking *L. caffeina* or *L. coffeella* on coffee. Specimens of *M. leucopterae* were also obtained by Ritchie from a *Leucoptera* on *Pavetta ternifolia* in Nyakato, Bukoba, but there is nothing to show that he found this leaf miner on coffee also, and it seems possible that the host species was another species of *Leucoptera*. Some mined leaves from coffee in Arua, Western Uganda, were sent to the writer by Mr. A. R. Melville, and these appeared from the eggs present to be *L. caffeina*, but no adults were obtained ; Mr. W. V. Harris, in a personal communication, stated that *L. caffeina* is found on coffee in Mbeya, basing his identification on the eggs and mines only. Some mines seen by the writer at Dalle in Abyssinia appeared to be *L. caffeina*, but no opportunity occurred to confirm this.

In the Kilimanjaro coffee growing areas serious losses from leaf miner were recorded by Ritchie (1935), who noted that two species were concerned, an observation which was also made by Kirkpatrick (1935).

In early 1937, the writer commenced a study of these two species on Kilimanjaro, which continued until his release for military duties in 1940. In late 1937, Mr. T. W. Kirkpatrick also commenced work on the same subject at Amani, and a division of the work was arranged ; very close cooperation was maintained until he also was released for active service.

Ferrière (1936) has given a list of 16 Hymenopterous parasites of the two *Leucoptera* spp. on coffee, of which ten were new species, mostly described from material received from Ritchie ; unfortunately it is not clear in many cases from which species they were obtained. The first object of the work was therefore to determine this, and to follow out as far as possible fluctuations of population affecting both species of *Leucoptera* and their parasites, in the hope that such studies would yield a clue to the causes of such fluctuations.

Life-history.

The life-history of *L. coffeella* has been described by Box (1923). Mr. Kirkpatrick has kindly placed at my disposal figures on the bionomics of this insect obtained at Amani, Eastern Usambaras, at an altitude of 3,000 feet. The rate of development from oviposition to emergence of adult at average temperatures from 19°C. to 25°C. for both *L. coffeella* and *L. caffeina* is shown fig. 1.

Nearly all adults emerge from the pupae between 06 hours and 08 hours. They remain quiescent, usually on the lower side of a coffee leaf, until about 15·45 to 16·00 hours, when they begin to fly or crawl round in search of a mate; the males more actively than the females. The male fans the female with his wings for from a few seconds up to a minute or more preparatory to copulation, which lasts until dusk or shortly after. Occasionally they may separate for a time and subsequently re-copulate. Copulation is repeated most evenings, though one copulation is sufficient for the fertilisation of eggs laid during the following twelve days. Copulation is only very exceptionally observed at other times of the day.

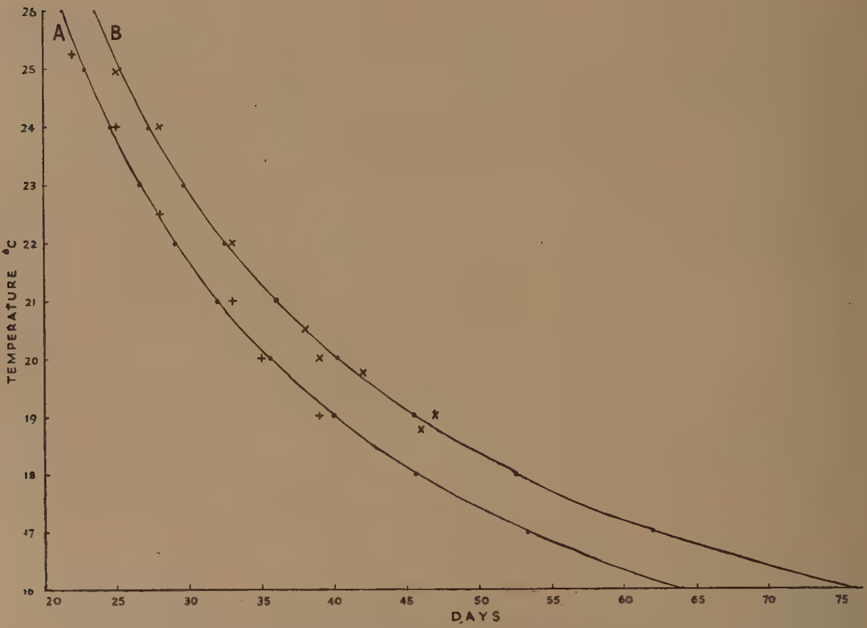


Fig. 1.—Developmental period from oviposition to emergence of adult. A, *L. caffeina*; B, *L. coffeella*, ; X, observed data.

Theoretical hyperbolae, as calculated from mean of observed data, based on Threshold of Development, A. 11·0°; B. 11·6° and Thermal Constant A. 320°; B. 341°.

Oviposition takes place during the night. Virgin females may lay a few eggs, but these do not hatch. Eggs are almost invariably laid on the upper surface of the leaf unless the position of the leaf is reversed, when about equal numbers of eggs are laid on each side. Females will not lay, however, unless given access to the lower surface, though they will lay normally on the upper surface if the lower surface is covered with a thick layer of soft paraffin wax or vaselene. They are laid singly or in small groups, not orientated with regard to a vein.

After emergence of the adult there is a pre-oviposition period of variable length, with an average of 2·1 days (min. 1, max. 14). An average of 75 eggs (min. 2, max. 154) was laid over an average period of 13·4 days (min. 1, max. 38), and there was an average post-oviposition period of 4·5 days (min. 0, max. 20) before death. All these figures were obtained from observations on 100 females fed with sugar solution and continuously supplied with males. There was a close correlation between duration of egg laying and number of eggs laid, though the number of eggs laid by individual females falls off with age. The daily total of eggs laid by all the females, and also the number of surviving females and the number of females actually ovipositing, is shown in fig. 2.

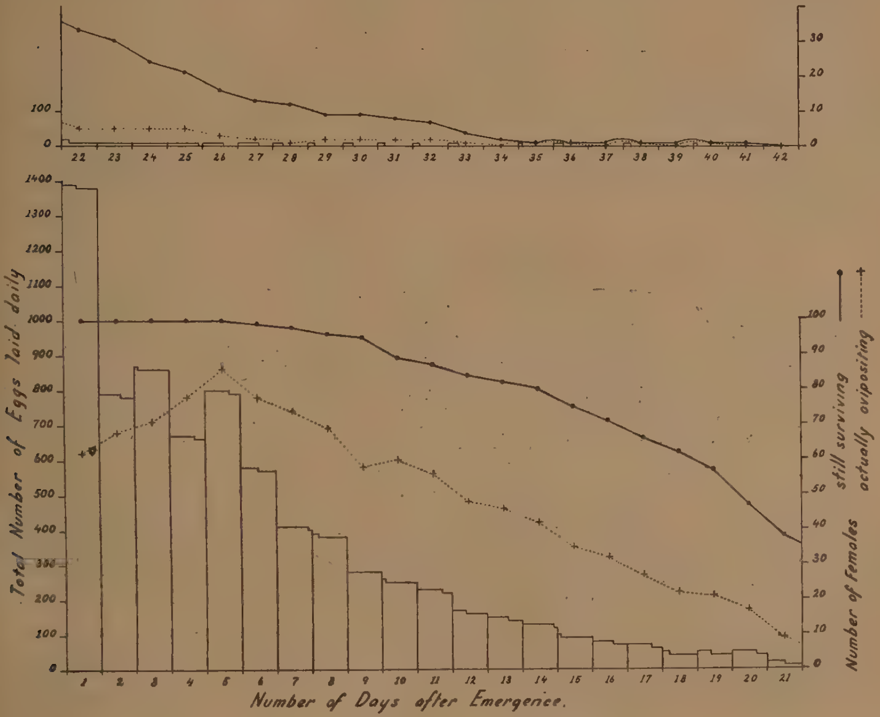


Fig. 2.—The number of eggs laid daily by 100 fertilised females of *L. coffeella.*

Although the longest period of oviposition observed is 38 days, the majority of eggs are laid early in adult life ; 49·2 per cent. were laid in the first four days, and 78 per cent. in the first eight days after emergence.

Females fed only on water lived a shorter time and laid fewer eggs than females fed on sugar solution ; the average number of eggs in this experiment being 42·0 for sugar-fed females, laid over a period of 17 days, and 27·5 eggs for females fed on water only, laid over 5 days only. All the water-fed females were dead by the 13th day.

It appears that oviposition may be greatly affected by conditions of larval development. Larvae collected at Burka Estate, Arusha, which emerged at Amani, were allowed to oviposit under the same conditions as adults obtained from larvae collected at Amani. Burka Estate has a much drier climate than Amani, and a much greater diurnal range of temperature ; situated at about 4,400 feet, it probably has an average temperature lower than that of Amani. The females from Burka lived as long as those from Amani, and individuals that oviposited laid as many eggs per night, but a larger percentage of the Burka females laid on only a very few nights or not at all, thus bringing the average eggs per female down to 7·6, compared with 27·8 for the Amani females.

The duration of the egg stage is fairly constant for the same temperatures. The following figures were obtained in the laboratory :—

Approximate average air temperature °C.	Time in days
24·6	5
24	5½–6
22	6–7
21	7–8
20·7	8–9
19·5	9–10

In the laboratory about 90 per cent. of eggs hatch ; failures to hatch are more frequent in eggs from females which have been previously laying over a long period.

The eggs turn pale brown about one day before hatching. Two to four days after hatching, the mines made by each larva of a group, which at first are roughly circular, join up to form a common mine. There is no evidence of cannibalism, even when mines containing young and old larvae coalesce.

The number of larval instars appears to be four. The excreta is pasted to the upper surface of the mine except near the edges. The average area of leaf-mine per larva is 50 square millimetres. Larval mortality in cages in the laboratory is about 25 per cent. Heavy rain has no effect on larvae in the mine unless it is split, or has an exit hole, when it may fill with water and the larvae in it may be drowned.

The duration of the larval stage is rather variable ; the following figures are the minimum periods observed in the laboratory :—

Shade temperature (average) °C.	Days
24·0	13
22·0	18
20·5	18
20·0	20
19·5	16 (one only, next shortest 21)
19·5	21
19·0	24

Outside, on unshaded coffee, larval development took one to three days longer than the above minimum times.

The following variations in larval periods for larvae hatching from eggs laid on the same day were recorded :—

Shade temperature (average) °C.	Days
20·5	18–23
19·5	16–31

The full-grown larvae leave the mine by a semicircular slit in the upper surface, usually near the edge. Several may emerge from the same slit. They usually drop by a thread to a lower leaf or to weeds below the bush. Larvae most usually spin on the underside of a coffee leaf and pupae tend to be more numerous on the sheltered side of exposed bushes. Spinning is completed in about 12 hours or a little less.

The prepupal stage, from the completion of the cocoon until the casting of the larval skin, lasts 30–36 hours.

Of individuals that spin on the same day, females emerge one day (occasionally two) earlier than males.

Average temperature °C.	Duration of pupal stage (days)	
	♂♂	♀♀
22	7	6
21	7–8	6½–7
20·5	8	7
19·5–20	11	9–10

Under laboratory conditions 97–98 per cent. of larvae that spin cocoons emerge as moths. Larvae bred on robusta coffee, however, showed a high prepupal mortality.

The pupal exuviae remain completely within the cocoon. Box (1923) states that the empty pupal skin is extruded, but this has only been seen when the pupa has been parasitised.

The total life-cycle, from egg to emergence of adult, is given in the following table :—

Average temperature °C.	Approximate average period (*i.e.*, mode)
24	27–30 days
20·5	35–40 ,,
20	40–45 ,,
19·5	45–50 ,,

On the mortality rates given above, the rate of multiplication should be about ×25 each generation (1♂+1♀ lay 75 eggs; 10 per cent. egg mortality=67·5 larvae; 25 per cent. larval mortality=50·6 pupae; 3 per cent. pupal mortality=49·1 adults, with sexes equal). In an experiment in which seven females and ten males were placed on a coffee tree covered with a muslin cage, from which all mined leaves had previously been removed, something approaching this rate of multiplication was actually realised. After 78 days (just over two generations) the number of larvae which had been produced was estimated, from the total area of leaf surface mined, as approximately the same as the theoretical figures, 9,100 (*i.e.*, $7 \times 50 = 350$ in the first generation, and $\frac{350}{2} \times 50 = 8{,}750$ in the second generation).

At the time of this estimate numerous adults of the second generation and very numerous eggs of the third generation were present; this generation almost completely failed to mature since nearly all the remaining leaves on the tree were shed owing to excessive damage before the larvae were full grown.

It is interesting to note, however, that from field studies at Lyamungu (see below) the rate of increase, even when parasitism is very low, does not approach this figure; this may be linked with the much lower average number of eggs laid per female such as has been shown to exist with adults from Burka above. From the figures given above, it may be inferred that a parasitism of over 90 per cent. is necessary to keep the host population from increasing, even when allowance is made for the habit of many parasites of stinging all larvae in a mine but laying only a small number of eggs. From field studies, however, it appears that parasitism of about 50 per cent. only is sufficient to retain the balance between host and parasites.

Field Studies—Methods.

L. coffeella exists most commonly on unshaded coffee, though it is found also on shaded coffee, where its presence may be masked by *L. caffeina*, which is often epidemic in such situations. Five trees were selected in a typical unshaded area, and five others in a typical heavily shaded area, the two plots being about 200 yards apart. All mined leaves were stripped from these trees. Subsequently all mined leaves were stripped each fourteen days, the leaves from each tree being kept separate. The eggs of *L. coffeella* and *L. caffeina* are easily distinguished; the former are smaller and more pointed, and are laid in groups at random on the leaf, while those of *L. caffeina* are larger and rounder, and are laid in rows of from two to fifteen along the veins of the leaf. Occasionally, however, unhatched eggs escaped notice, and *coffeella* eggs laid on a part of the leaf which had become involved in a *caffeina* mine were easily missed. The number of eggs of each species was recorded, and the mines separated into four groups; *L. coffeella* from unshaded coffee; *L. coffeella* from shaded coffee, *L. caffeina* from unshaded coffee, *L. caffeina* from shaded coffee. These were placed in separate parasite emergence cages, after the removal of all cocoons from the leaves. The cages were kept for six weeks, it having being found that no further insects emerged after this period. Occasionally, when the number of leaves to be dealt with was very large, all the mined leaves were stripped and counted, and 200 (in 1938) or 400 (in 1939 and 1940) were examined in detail as above, the total numbers being obtained by calculation.

Moths and parasites emerging from these cages were recorded daily. At the end of the six-week period the cages were opened and searched, the adults found in the cages, which had failed to come to light, being also recorded.

Pupae were collected from adjacent similar trees and placed separately in one-inch gelatine capsules; these were also kept for six weeks. It is possible to distinguish *L. coffeella* and *L. caffeina* pupae by the fact that in *caffeina* the pupal antennae extend beyond the third legs, whereas in *coffeella* they do not.

There is, unfortunately, no ready means of distinguishing larvae which have left the mine and spun up and have then died, as is the case with a great many of the parasitised larvae, so that studies of pupal parasitism where both species exist together are difficult, but *L. caffeina* is almost absent in unshaded coffee, and under shade, during an outbreak of *caffeina*, *coffeella* amounts to only about one-tenth of the *caffeina* population. It is thus possible to draw broad conclusions on the parasitism of cocoons of the dominant species, but more difficult to do so on that of the secondary one in each case.

Leucoptera coffeella **in unshaded Coffee.**

Egg counts. Fig. 3 shows the number of eggs of *L. coffeella* per tree in the unshaded experimental plot during 1938, 1939 and 1940. In 1938 a severe outbreak of *L. coffeella* took place on the plot, in 1939 there was hardly any outbreak and in 1940 a mild outbreak took place.

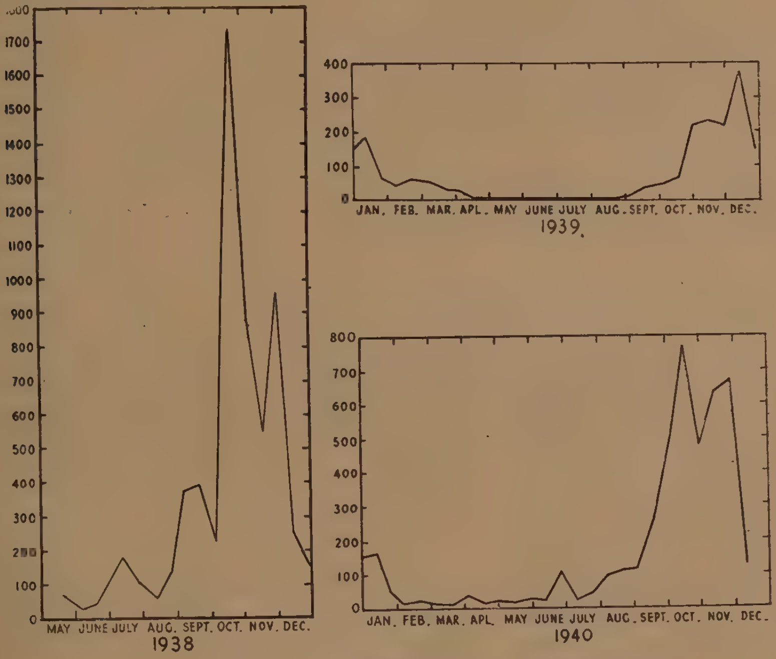


Fig. 3.—*L. coffeella.* Number of eggs per tree. Unshaded coffee.

It will be noted that in each year the number of *L. coffeella* begins to rise between May and June and reaches a peak between October and December, thereafter dropping rapidly again. Fig. 4 shows the common logarithms of the same figures with the values for the observation plus and minus the standard error (*Standard Deviation*) shown as points above and below each observation. It is evident from fig. 3 that the number of eggs reached minimum in May, 1939 (0·4 per tree), and March 1940, (11·4 per tree).

Egg parasites. Unless a Mymarid, *Polynema* sp., which emerged occasionally from collections, is a parasite of the eggs, and there is no other evidence for this, no egg parasites have been recorded. Kirkpatrick has recorded two species of Thysanoptera sucking eggs of both *L. coffeella* and *L. caffeina*.

Larval parasites. Fig. 5 records the larval parasitism of *L. coffeella* in unshaded coffee. From the end of April to the middle of August, 1939, and from the middle of February to the beginning of April, 1940, no insects were hatched from the very few mines obtained.

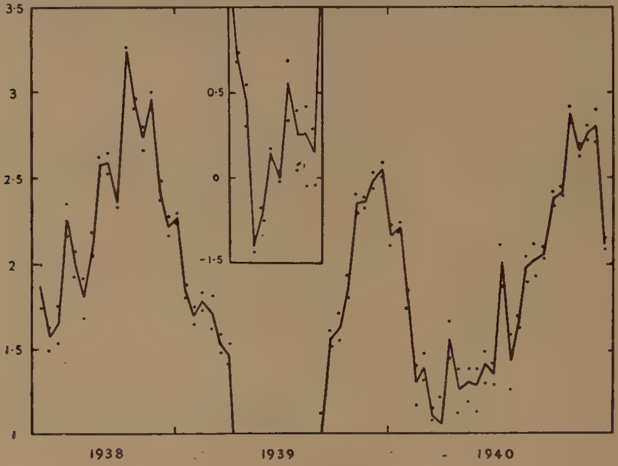


Fig. 4.—*L. coffeella.* Logarithms of number of eggs per tree. Unshaded coffee.

Only the two important parasites, *Atoposoma variegatum*, Masi, var. *afra*, Silv., and *Apanteles bordagei*, Giard, are shown. Other parasites recorded as emerging from these collections, but never in sufficient numbers to affect the population very appreciably, are *Pleurotropis coffeicola*, Ferr., *Achrysocharella ritchiei*, Ferr., *Chrysocharis lepelleyi*, Ferr., *Elasmus leucopterae*, Ferr., *Teleopterus leucopterae*, Ferr., *Eulophus borboricus*, Giard (probably from pupae included in error), *Cirrospilus cinctiventris*, Ferr., Gen. nov. nr. " *Hormius* " and *Ageniaspis* sp.

Both *A. bordagei* and *A. variegatum* are known to be primary parasites and probably cannot be secondary.

From these figures it will be seen that larval parasitism follows the same pattern throughout each year. It was low in May, 1938, and May, 1940 ; it must have been low also in the same period of 1939, though no records were obtained, since the population of hosts was increasing rapidly and when records were again obtained, in August, parasitism was between 0 and 20 per cent. A rise of larval parasitism took place in the second half of each year. *A. variegatum* was leading in 1938 and 1940 and *A. bordagei* in 1939, when it reached the figure of nearly 30 per cent. in early October. *A. variegatum*, however, finally took the lead in each year, and where records are available, namely in April, 1939, and February, 1940, appears to compete successfully with *A. bordagei* for the very few *coffeella* larvae left.

Very shortly after this larval parasitism falls. In 1939 two collections in April yielded only parasites ; thereafter, as has been shown above, the percentage parasitism must have fallen, though no adults were obtained, until, when figures were again obtained in August, 80 to 100 per cent. of larvae were escaping parasitism. In February, 1940, only 9 per cent. of the larvae were escaping parasitism, but from successive collections during April, 1940, 34, 62 and 85 per cent. of the larvae escaped parasitism.

Pupal parasites. Fig. 6 records the pupal parasitism in 1938, 1939 and 1940. It must be explained that by a pupal parasite is here meant a parasite that emerges from a host which has spun its cocoon ; from these may emerge not only parasites of the prepupa and true pupal parasites, but also endoparasites that attack the larva, but allow it to leave the mine and spin up and sometimes to pupate.

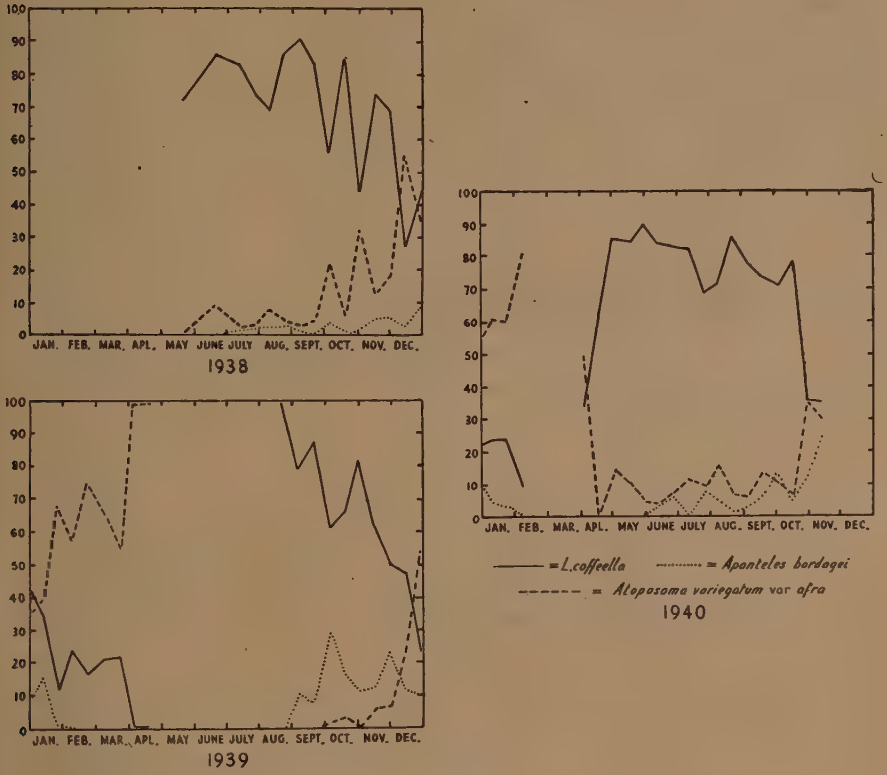


Fig. 5.—*L. coffeella.* Larval parasitism per cent. Unshaded coffee.

Collections of cocoons were begun in September, 1938, and were made rather irregularly until January, 1939, after which they were made weekly until August, 1939, from which date they were made twice weekly. In figure 6, the percentages for 1939 and 1940 have been averaged over fortnightly periods to smooth out variations caused by small numbers.

The most important parasites are the one determined as Gen. nov. nr. *Hormius* (hereafter referred to as " *Hormius* "), *P. coffeicola* and *A. bordagei*. " *Hormius* " is a primary parasite attacking larvae, prepupae and pupae ; *P. coffeicola* may be primary, attacking larvae in the mine which usually spin up, or it may be a hyperparasite on many other parasites, such as " *Hormius* " and *A. bordagei*, or a superparasite with *Ageniaspis* sp. or " *Hormius* ". *A. bordagei* is primary only, attacking larvae, which then spin up, and possibly prepupae.

Other parasites obtained from pupal collections are *Ageniaspis* sp. (Primary), *Hemiteles* sp. (Primary), *Eulophus borboricus*, Giard (Primary, and ex "*Hormius*" and *A. bordagei*), *Achrysocharella ritchiei*, Ferr. (Primary), *Syntomosphyrum* sp. (ex *A. bordagei*), *Pleurotropis* sp. (ex *A. bordagei* and "*Hormius*". Possibly also primary).

In each case the identity of the host species has been confirmed by dissection.

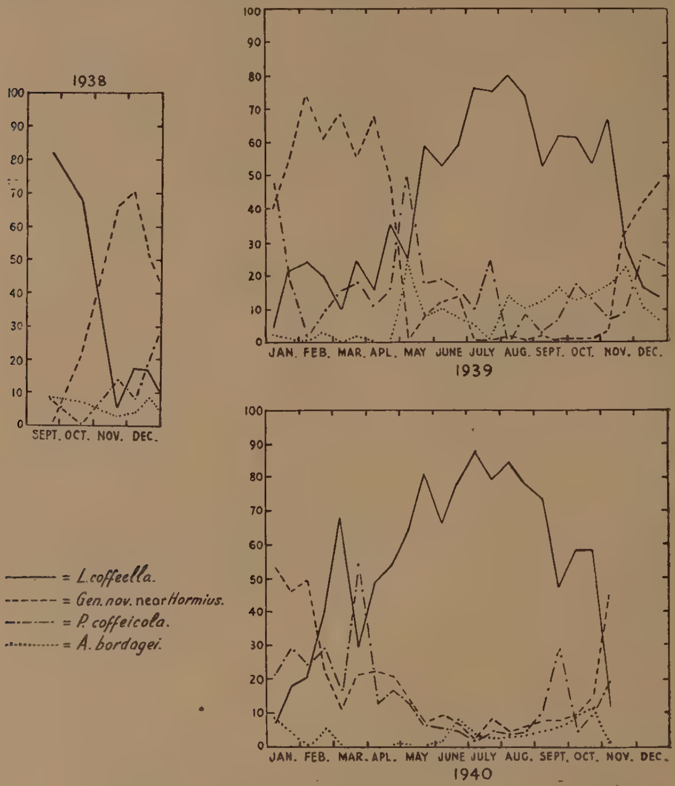


Fig. 6.—*L. coffeella.* Pupal parasitism per cent. Unshaded coffee.

In each year the percentage of pupae escaping parasitism fell from September onwards, and very rapidly in November. Subsequently from January to July it rose fairly steadily from below 10 per cent. to between 70 and 80 per cent. It seems clear that this annual rise is not due to climatic factors, since it begins in the hot dry months, continues throughout the rains, and reaches its peak in the coldest months of the year.

At the time of highest percentage parasitism, "*Hormius*" is the most important parasite ; it reached nearly 50 per cent. at the end of 1939 and 1940, and 70 per cent. at the end of 1938 and early 1939. Later the percentage of this parasite fell, whilst *P. coffeicola* rose to about the 50 per cent. level in May, 1939, and March, 1940, falling again in the following three months to below the 10 per cent. level. *A. bordagei* does not appear to have been of great importance.

It is interesting to note that the collapse of the parasitism by "*Hormius*" coincided in both 1939 and 1940 with the end of the fall in egg counts. It also coincided with the suppression of the larvae by *A. variegatum*. It appears, therefore, that as the outbreak comes under control, there is fierce competition for the remaining hosts. "*Hormius*" is a very successful parasite while there are plenty of hosts, but *A. variegatum* attacks larvae, while "*Hormius*" mostly attacks prepupae and pupae. As the host comes under control there are large numbers of both species of parasite present, but if few larvae are escaping parasitism by *A. variegatum* and other parasites, it seems likely that pupal parasites may spend a disproportionate amount of time investigating the very large numbers of empty cocoons left from previous generations of the host, and have little chance of finding the new unparasitised full ones. If this is so, then the percentage of true prepupal and pupal parasites is bound to fall. *P. coffeicola* has a better chance of survival since it can attack larvae in the mine or other parasites such as *A. bordagei* and "*Hormius*".

Leucoptera coffeella **in shaded Coffee.**

Egg counts.—Fig. 7 gives the number of eggs per tree on the shaded plot. It is clear that the population of *L. coffeella* in these conditions is not subject to the great fluctuations exhibited in unshaded conditions, the number varying from 4 to 304 per tree. The same tendency to increase towards the end of the year is shown, with an out-of-season peak in July 1939.

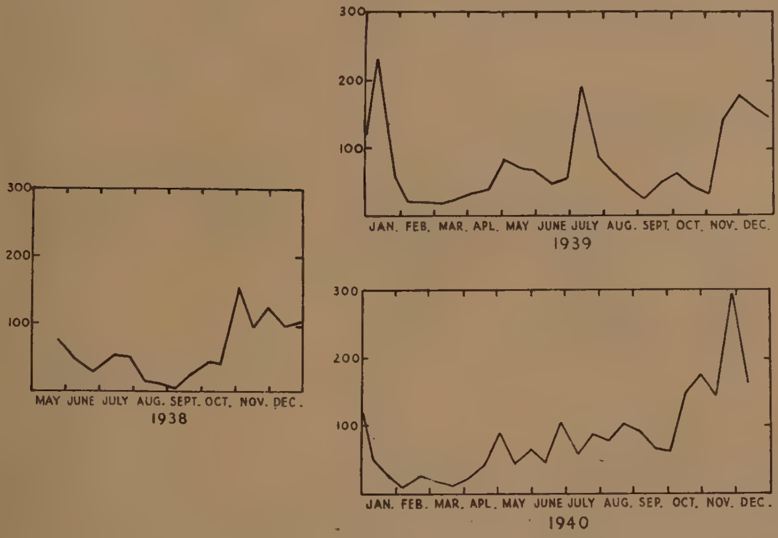


Fig. 7.—*L. coffeella*. Number of eggs per tree. Shaded coffee.

In 1940, the rise, though fluctuating, continues from February to November; the fluctuations showing some similarity to those of *L. coffeella* in unshaded coffee in 1938.

Larval parasites.—Fig. 8 gives the percentage figures for the emergence from these collections of *L. coffeella* and *A. bordagei*, which is the only consistently important parasite of the larvae of *L. coffeella* under shade. It is seen from the diagrams that the fluctuations in percentage emergence between *A. bordagei* and its host are very closely complementary; the host and parasite together account for 80 to 100 per cent. of all emergences in the great majority of collections. The collections between 3rd November, 1939, and 11th January, 1940, were not separated into host species, and the percentages of the parasites that may have hatched from *caffeina* or *coffeella* larvae are doubtful, this probably making the percentage of *A. bordagei* which is shown lower than it actually was. *A. bordagei*, however, does not parasitise *L. caffeina*.

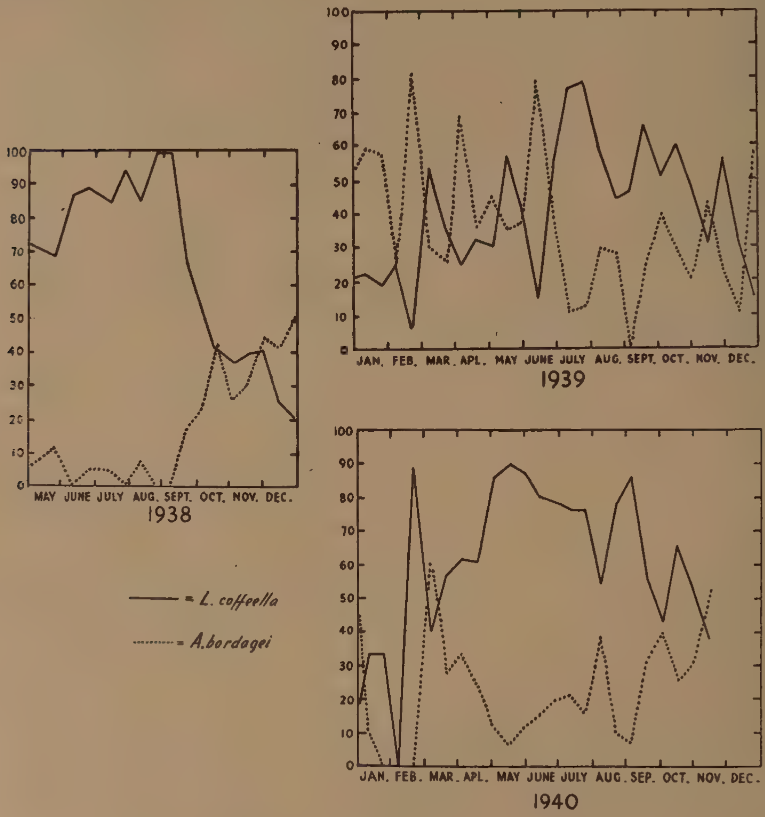


Fig. 8.—*L. coffeella.* Larval parasitism per cent. Shaded coffee.

Other parasites appearing from larvae are *Elasmus leucopterae*, Ferr. (occasionally common, but mainly absent), *Ageniaspis* sp. (rare as a rule, but sporadically common), *Pleurotropis coffeicola*, Ferr. (present in small numbers throughout), *Achrysocharella ritchiei*, Ferr. (fairly common at the end of 1939, otherwise rare), *Chrysocharis lepelleyi*, Ferr. (very rare), *Atoposoma variegatum*, Masi, var. *afra*, Silv. (very rare), *Tetrastichodes leucopterae*, Ferr. (occasionally common), *Closterocerus africanus*, Wat. (very rare), *Derostenus coffeae*, Ferr. (very rare), and *Hemiteles* sp. (very rare).

Pupal parasites.—As in the previous discussion of pupal parasitism, all parasites that emerge from cocoons collected in the field are included, whether they oviposit in larvae, prepupae or pupae.

The difficulty of separating cocoons of *L. coffeella* and *L. caffeina* has already been mentioned. While this was unimportant as regards *L. coffeella* in unshaded coffee, since *L. caffeina* was rare in these circumstances, the problem is very much more difficult when *L. coffeella* is being studied under shade conditions, since *L. caffeina* during part of the year is epidemic under shade, whilst *L. coffeella* is not, and *L. caffeina* was almost always more numerous than *L. coffeella* during the period of the observations in this shaded plot. It was found, however, that if cocoons are collected from the trees, a very large proportion of them are *L. coffeella*, while those from leaves which have fallen to the ground are almost entirely *L. caffeina*. Caution must be exercised in drawing conclusions from these figures owing to the possibility of the proportion of *L. caffeina* included in the collection affecting the percentages considerably. Fig. 9 shows the percentage of the more important parasites from these collections. Collections were made somewhat irregularly from May, 1938, to June, 1939, but thereafter twice weekly. The figures from July, 1939, onwards are obtained by combining all the collections each fortnight.

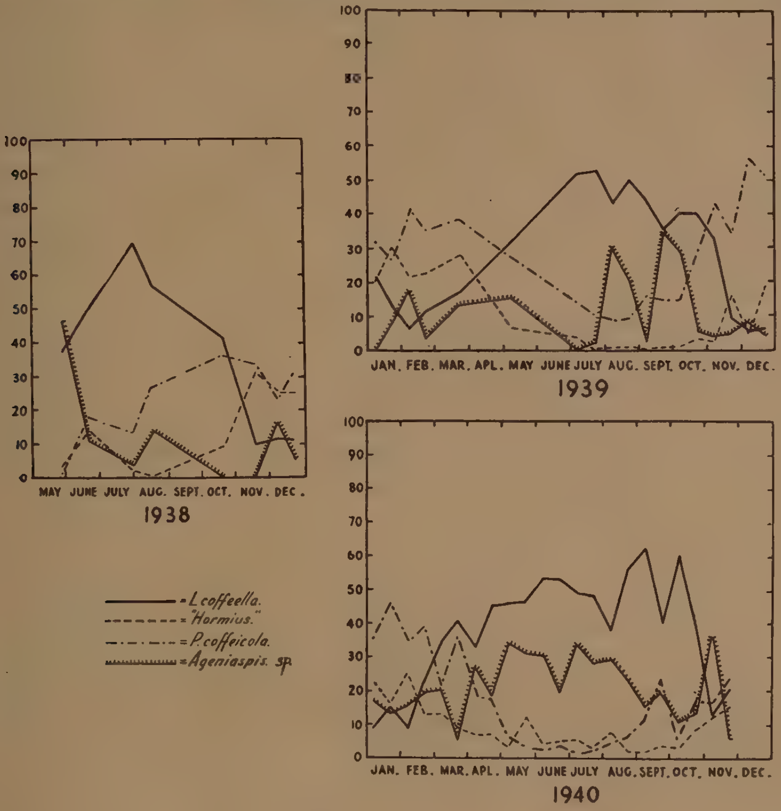


Fig. 9.—*L. coffeella*. Pupal parasitism per cent. Shaded coffee.

A single *Leucoptera* larva normally produces several adults of *Ageniaspis* sp., any number from three to seven parasite pupae being found in a single larval skin. The average number of adults actually hatched in the laboratory was 3·5 per cocoon though the number of cocoons per host was usually five. The percentage figures for this parasite therefore tend to exaggerate its true importance, since only a third to a quarter of this percentage of *Leucoptera* larvae is destroyed. On the other hand, the importance of many of the larval ectoparasites is minimised, since many of these sting all the larvae in a mine but lay only a very few eggs.

Two parasites that appeared regularly, *A. bordagei* and *E. borboricus*, are not shown in the figures. *A. bordagei* was never completely absent, and averaged 6·5 per cent. of all collections (highest 20·7, lowest 2·2). *E. borboricus* was usually present, though absent from twelve collections, and averaged 3·8 per cent. (highest 16·8).

The general picture is much the same under shade as in unshaded coffee, the host largely escaping parasitism from May or June to October and being heavily parasitised after November, but under shade *P. coffeicola* is almost always more important than "*Hormius*", and *Ageniaspis* sp. becomes of very considerable importance, especially in the months when the percentages of other parasites fall to a low level.

Other parasites obtained from *L. coffeella* cocoons occasionally are *Syntomosphyrum* sp. (ex *E. borboricus*, *Ageniaspis* sp., "*Hormius*" and *A. bordagei*), *Hemiteles* sp., *Pleurotropis* sp. (ex *Ageniaspis*, *E. borboricus* and *A. bordagei*) and *Cirrospilus longifasciatus*, Ferr.

Occasionally both *E. borboricus* and *Ageniaspis* sp. emerge from the same pupa, as do *P. coffeicola* and *Ageniaspis* sp.

It has been confirmed by dissection that these were obtained from *L. coffeella* as the original host.

Bionomics of Parasites.

The following notes on the parasites of *L. coffeella* are largely taken from notes by Mr. Kirkpatrick.

Apanteles bordagei.—Primary endoparasite of *L. coffeella*. No record from *L. caffeina*. Distribution: E. Usambaras, Moshi, Arusha, Kiambu, Reunion. Only females known from all localities except Kiambu, where one out of four specimens appeared to be a male. Normal reproduction parthenogenetic. Oviposits in larvae in mine; host larva almost invariably spins a cocoon. Twenty-four to thirty-six hours after host cocoon spun, parasite larva leaves host, pushes out empty larval skin from cocoon, and itself forms a thin parchment-like cocoon inside the *Leucoptera* cocoon. Life-cycle (egg to emergence of adult) 14 days (February) to 25 days and upwards (July). Adults fed on syrup lived to 15 days. All the Chalcids associated with *Leucoptera*, whether ecto- or endo-parasites, act as hyperparasites, since they do not reject larvae already parasitised by *A. bordagei*.

Atoposoma variegatum var. *afra.*—Primary ectoparasite on *L. coffeella* and *L. caffeina*. Stings and paralyses larvae in mine, but not always all larvae in a mine. Female feeds at the stinging puncture, through which usually quite a large drop of liquid exudes. Eggs laid loose in the mine, up to five eggs per larva, though sometimes only one egg laid. Pupates in mine, very loosely if at all attached by larval skin. Life-cycle 13–29 days. As many as three parasites may develop from one larva. Adult lives up to 90 days, and eggs may be laid up to 61 days from emergence. Pre-oviposition period 4 days. Unfertilised females oviposit readily, producing only males; fertilised females produce about 62 per cent. females. Hyperparasites are *P. coffeicola*, *Closterocerus africanus* and *Teleopterus violaceus*.

Elasmus leucopterae.—Primary ectoparasite of *L. coffeella* and *L. caffeina*. Stings host in mine, usually stings all larvae in mine, but frequently misses some. Up to three parasites may develop in one mine. Life-cycle 14–24 days. Adults live 65 days, and lay up to 63 days in laboratory. Hyperparasites are *P. coffeicola* and *Teleopterus violaceus*.

Pleurotropis coffeicola.—Primary and secondary endoparasite on *L. coffeella* and *L. caffeina*. Not very common as primary on *L. coffeella* on arabica coffee at Amani, but common as primary on robusta coffee. Life-cycle 18–38 days. Adults live up to 58 days, and oviposit for 48 days. Secondary on *Eulophus* sp., *Elasmus leucopterae*, *Cirrospilus cinctiventris* and *Atoposoma variegatum*. A female, whether it developed as a primary or secondary, will oviposit in a primary or secondary host indifferently. Virgin females oviposit readily, producing males. When primary, the host usually pupates. No hyperparasites recorded when primary. *Chrysocharis* sp. recorded ex *P. coffeicola* ex *Eulophus* sp.

Achrysocharella ritchiei.—Primary endoparasite on *L. coffeella* and *L. caffeina*. Stings and oviposits in larvae in mine. Does not sting without egg laying. Will lay up to three larvae in one mine. Host feeds for 1–2 days after being stung. Pupates in mine. Life-cycle 15–21 days. Adults lived and oviposited up to 34 days. Virgin females oviposit readily, producing males. Hyperparasite, *Closterocerus africanus* (probably).

Chrysocharis lepelleyi.—Primary endoparasite on *L. coffeella* and *L. caffeina*. Stings larvae in mine, usually but not always all the larvae in a mine. Occasionally lays two eggs in a host, but only one develops. Host larva immediately and almost completely paralysed. Will not oviposit in larvae less than two-thirds grown. Life-cycle 20–38 days. Adults have lived 95 days and laid over 71 days. Unfertilised females obtained from Kenya produced only males, but unfertilised females from the Usambaras were bred through seven generations without producing a single male. Hyperparasites are *Closterocerus africanus*, almost certainly, and any ectoparasitic Chalcid may be a facultative hyperparasite.

Tetrastichodes leucopterae.—Primary ectoparasite on *L. coffeella* and *L. caffeina*, occasionally secondary on *Eulophus* sp., and sometimes cannibal. Males are rare. All larvae in mine stung as a rule; they are not completely paralysed. Will lay up to 12 eggs in a mine, but 2–4 most usual. Two parasites may develop from one host. Life-cycle 15–28 days; adults have survived 63 days and oviposited for 46. Parthenogenetic and thelyotokous, but males exist, though rare. Hyperparasites are *Closterocerus africanus* and *Teleopterus violaceus*.

Closterocerus africanus.—Secondary endoparasite on *Eulophus* sp., *Atoposoma variegatum*, *Tetrastichodes leucopterae*, and almost certainly on *Chrysocharis lepelleyi* and *Achrysocharella ritchiei*. Never primary. Primary host may be *L. coffeella* or *L. caffeina*. Life-cycle 21–30 days (June to August). Adult has survived 85 days and oviposited over 53 days.

Derostenus sp. (*? coffeae*).—Primary endoparasite on *L. coffeella* and *L. caffeina*, and on *Acrocercops bifasciata*. Will oviposit in up to three larvae in one mine. Pupates in mine. Failed to mate in captivity, and produced only males. Life-cycle (males only) 14–19 days; female has lived 45 days.

Teleopterus violaceus.—Secondary endoparasite on *Eulophus* sp., *Elasmus leucopterae*, *Cirrospilus cinctiventris*, *Tetrastichodes leucopterae*, *Atoposoma variegatum*. Usually only found where *L. coffeella* is primary host. Always leaves host to pupate; host often pupates before parasite emerges. Life-cycle 16–32 days. Females seldom survive more than 25 days. *Teleopterus violaceus* is also a primary endoparasite of *Melanagromyza* sp.

Eulophus borboricus.—Primary endoparasite on *L. coffeella* and probably *L. caffeina*. Normally oviposits in cocoon before or just after pupation. Eggs laid on host, not inserted. Occasionally investigates larvae in mines and apparently stings them, possibly for feeding at the puncture, but there is no evidence that eggs are laid in larvae; such larvae die before spinning. If a larva is investigated before spinning is completed, it may escape by leaving the half finished cocoon and spinning another. Inclined to superparasitism, when no parasites survive. Pupates more or less inside empty pupa of host ; adult bites circular hole in cocoon. Unfertilised females will lay, producing males. Life-cycle 13–20 days (May to June). A female has lived 69 days in the laboratory, but most die in 30 to 40 days. Hyperparasite, almost certainly *Pleurotropis coffeicola*.

Cirrospilus cinctiventris.—Primary and secondary ectoparasite, on *L. coffeella*, *L. caffeina*, *Acrocercops bifasciata*, *Eulophus* sp., *Tetrastichodes leucopterae* and on *Achrysocharis* sp. on *Melanagromyza*. Will not oviposit on full grown larvae, requiring half grown or smaller ones. Often does not sting all larvae in a mine ; two sometimes, and once three, developed from a single mine. Life-cycle 10–26 days. Females have survived 2½–3 months, and laid eggs over 79 days. The same female will lay as a primary or secondary.

Three main varieties known :—(i) Type form—abdomen with two black transverse stripes ; (ii) Stripes interrupted at mid dorsal line (commonest form at Amani) ; (iii) Abdomen all orange, no dark markings (scarce).

Females of (i) have produced males of (ii) but (iii) always breeds true, and will not pair with males of (ii).

Gen. nov. nr. *Hormius*.—Primary endoparasite on *L. coffeella* and *L. caffeina*. Will sting large larvae in mine, prepupae in cocoon, or pupae, though possibly for only a short period after pupation. The host probably always pupates. Larva leaves pupa of host and pushes it out of cocoon, then spins a cocoon similar to that of *A. bordagei*, though somewhat more silky. Adult emerges by an irregular round hole in host cocoon. Unfertilised females produce only males. Apparently reject hosts already parasitised by *A. bordagei*. Life-cycle 13 to 16 days. This species was not found by Kirkpatrick in the Usambaras in 1937–1940, but a single specimen was obtained by the writer at Amani in 1947.

Eulophus sp.—Primary ectoparasite of *L. coffeella* and *L. caffeina*. May be secondary if host already attacked by endoparasite. Stings host in mine, females feed on moisture exuding through the puncture, and males sometimes investigate mines with females and may also feed thus. All larvae in mine usually stung. Sometimes up to three eggs laid loose in mine ; first larva to hatch sucks any other eggs present, and larvae are cannibalistic. One host is sufficient for development, though parasite may suck three or more. Full grown larvae preferred for oviposition. Life-cycle 12–27 days, one day longer for females than males. Egg 1–2½ days, pupae 6–15 days. Females have lived up to 77 days. Hyperparasites are *Pleurotropis coffeicola*, *Closterocerus africanus*, *Teleopterus violaceus*, *Achrysocharella* sp., *Achrysocharis* sp., *Cirrospilus cinctiventris* and *Tetrastichodes leucopterae*.

This species was not found in the Kilimanjaro area, but as it is a very effective parasite in the Usambara area, efforts are being made to introduce it.

The Dominant Stage in *L. coffeella*.

An interesting observation on the 1938 outbreak, which was the only serious one, appears to be that the host was present in what may be described as the " dominant stage ". By this is meant a condition where a great preponderance of the individuals at any one time are in the same stage of development ; it is comparable to what was described by Taylor (1937) for *Promecotheca reichei*, Baly, as the " one stage " condition. The term " one stage " can hardly be used to describe the present case, if this is intended to mean that all the individuals are in the same instar ; actually Taylor describes the " one stage " condition of *P. reichei* as having never more than three instars, and usually only two, present at the same time. Certainly the observations on *L. coffeella* described here did not indicate a condition so extreme as this. But fig. 4 shows that from May to November, 1938, the number of eggs collected fluctuated regularly and below any straight line or simple curve which may be drawn through the series of observations, and that these fluctuations are greater than the standard error. Further, in fig. 10, which shows the number of adults emerging daily from the collections from all five trees, it is clear that the emergences of adults are grouped round various dates, *i.e.* early June, early August, late September, mid-November and mid-December. The intervals between these dates correspond roughly with the length of the life cycle of *L. coffeella*, which varies from about 40 days in the cold season (June to August) to 32–35 days in the hot season (December to March). There is no evidence that this condition persisted in 1939, when no outbreak took place, though there is some evidence that it was present in a less marked form in 1940, when the outbreak was mild.

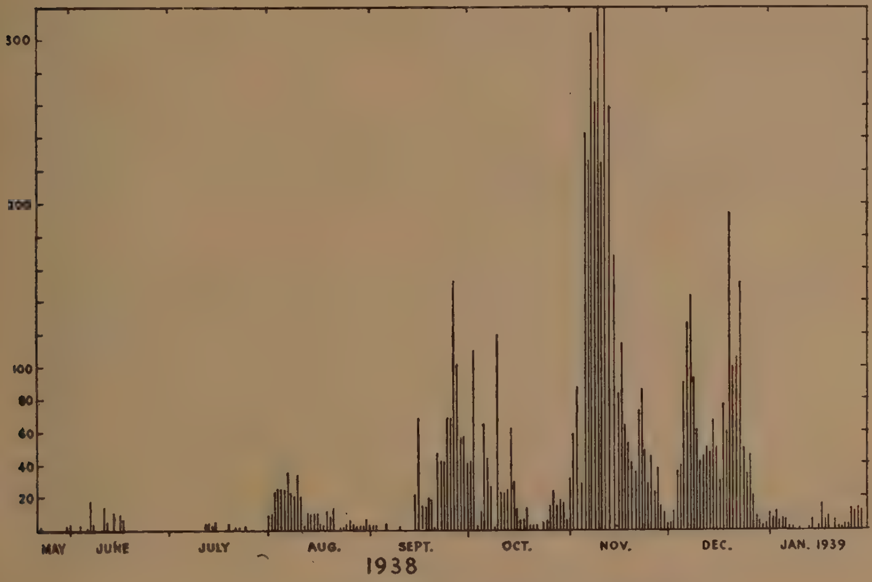


Fig. 10.—Daily hatchings of *L. coffeella*. · Unshaded coffee.

Box (1923) states that in Kenya " The writer has been able to find all stages of the insect at any time during the period of his studies " (December, 1921–May, 1922) " but occasionally one of them would predominate, owing probably either to local meteorological conditions or to the incidence of natural enemies ". It would thus appear that he met the same phenomenon in Kenya.

In fig. 5, marked regular fluctuations in the percentage parasitism of *L. coffeella* by *A. variegatum* in the year 1938 may be observed. These fluctuations correspond with the availability of larvae, as inferred both from the egg counts and the emergences of adults. There is naturally a fall in percentage parasitism when large increases of the numbers of larvae take place, followed by a rise in percentage parasitism as these larvae are progressively parasitised and few additions to the larval population are being made. A comparison of fig. 3, which shows the egg counts (made when the eggs have already hatched), with the percentage parasitism in 1938 by *Atoposoma* in fig. 5, shows that the four peaks in the former correspond with the four troughs in the latter, and *vice versa*. Regular periodic peaks in percentage parasitism, corresponding to the length of the host generation, may thus possibly indicate a dominant-stage condition of the host. A similar fluctuation of percentage parasitism by *A. bordagei* in 1939 may be observed in fig. 8, suggesting that *L. coffeella* may have been in a dominant-stage condition in the shaded coffee also in 1939.

It is tempting to speculate as to the effects of the dominant stage in an insect with such a comparatively short life history. It appears that the dominant stage is most marked during the period when the host is attaining epidemic numbers, although the rate of increase was actually slower when the dominant stage was marked, as in 1939, than it was in 1940, when the increase was rapidly brought under control by parasites. It might be that the dominant stage exerts a check on parasite activity by the periodic shortages of suitable stages for the parasites to attack, thus limiting for a time their effectiveness. Since, however, *Atoposoma*, for instance, can continue to oviposit in the laboratory over long periods (61 days), it is difficult to imagine what the nature of this check can be.

Summary.

Two leaf miners of coffee, *Leucoptera coffeella* and *L. caffeina* are present in the Kilimanjaro and Usambara districts of Tanganyika. The life-histories of *L. coffeella* and its parasites and a study made during 1937–40 of the populations of the host and its parasites in the Kilimanjaro area in both unshaded and shaded coffee are described. Some observations are made on the " dominant stage " of the host.

Acknowledgements.

Grateful acknowledgement is made to Professor T. W. Kirkpatrick, now of the Imperial College of Tropical Agriculture, Trinidad, for suggestions and criticisms during the course of the work, and especially for generously placing at my disposal all his notes and records on leaf miners and their parasites, made when he was Entomologist to the East African Agricultural Research Station, Amani, which have been freely drawn upon in the preparation of this paper.

I have also to record my thanks to the Commonwealth Institute of Entomology for the identification of specimens.

I would also like to record my appreciation of the work of Mr. Said Mohammed, African Laboratory Assistant, who assisted throughout the investigation and continued the collections unaided from June to December 1940.

References.

Box, H. E. (1923). Bull. ent. Res., **14,** pp. 133–145.

Ferrière, C. (1936). Bull. ent. Res., **27,** pp. 477–491.

Kirkpatrick, T. W. (1935). 7th Rep. E. Afr. agric. Res. Sta. Amani 1934–35 pp. 16–18.

Ritchie, A. H. (1935). Rep. Dep. Agric. Tanganyika 1934, pp. 73–83.

Taylor, T. H. C. (1937). The Biological Control of an Insect in Fiji. London, Imp. Inst. Ent.

Washbourn, R. (1940). Bull. ent. Res., **30,** pp. 455–462.

Wilkinson, D. S. (1936). Bull. ent. Res., **27,** pp. 385–388.

NOTES ON *PSEUDOCOCCUS COMSTOCKI* (KUW.) AND SOME ALLIED SPECIES (HOMOPTERA: COCCOIDEA), WITH DESCRIPTIONS OF THREE NEW SPECIES.

By N. S. Borkhsenius.

Zoological Institute of the Academy of Sciences of the USSR, Leningrad.

The determination of mealybugs closely related to *Pseudococcus comstocki* (Kuw.) has presented considerable difficulty in the past. Green (1921, p. 189) and Hough (1925, p. 52 and 1925a, p. 13) have given details of the distinguishing features for *P. comstocki* (Kuw.) and *P. maritimus* (Ehrh.) but the grounds given for the separation of the former from other closely related species are not fully satisfactory. The representatives of this group of mealybugs are becoming more and more widely spread throughout the world and of greater economic importance yearly. A correct designation of these insects is, therefore, of particular importance not only from the point of view of quarantine legislation but also of control measures. Research work on the morphological characteristics of species closely related to *Pseudococcus comstocki* (Kuw.) has been carried out on very large quantities of material from different parts of the world.

Material of *P. comstocki* (Kuw.) has been examined from different food plants from Tashkent and the U.S.A. (New York State). *P. maritimus* (Ehrh.) from different food plants has been seen from various regions of USSR, from Norway, Palestine, U.S.A., Argentina and Chile and *P. citriculus*, Green, from Citrus trees from Palestine. Large numbers of individuals of certain species were taken on bananas sent to Leningrad from Colombia and included three new species, the descriptions of which are given below, that are closely related to *P. comstocki* (Kuw.) and *P. maritimus* (Ehrh.).

As a result of these investigations certain conclusions were arrived at. The most consistent characteristics for the separation of the species of the genus *Pseudococcus*, related to *P. comstocki* (Kuw.) are: the number, and the presence or absence, of discoidal pores near the eyes (in the adult female and immature forms); the length of the body setae distributed on the margins of the dorsal ostioles and along the median dorsal line (in the adult female and immature forms); the number of the body setae and discoidal micropores, distributed near the mushroom-shaped ducts (in the adult female); the presence or absence, and if present, the distribution of tubular and mushroom-shaped ducts and of multilocular pores on the dorsal surface of the body (in the adult female). These new characteristics taken in conjunction with some others previously known, such as the structure of the cerarii, will greatly facilitate the recognition of these species.

Key to the Adult Females of *Pseudococcus comstocki* (Kuw.) and allied Species.

1 (8). Without multilocular pores on the dorsal surface of the body.

2 (5). With 2–8 discoidal pores near the eyes; body setae distributed along the posterior margin of the anterior dorsal ostioles up to 0·022 mm. long and those along a median dorsal line on the thorax up to 0·030 mm. long; hind coxae without translucent pores.

3 (4). With 2 or 3 discoidal pores near the eyes; mushroom-shaped ducts for the most part with one discoidal micropore and without body setae; without irregular discoidal pores; body setae along a median dorsal line on the thorax up to 0·021 mm. long..............................*P. maritimus* (Ehrh.)

row on the ventral surface of the abdominal segments. With 17 pairs of cerarii: the 1st–3rd cerarii of the cephalothorax with 3 spines about 0·013 mm. long, 3–4 auxiliary setae and 23–27 trilocular pores; the 4th, 5th, 7th–9th cerarii of the cephalothorax and the 2nd–4th abdominal cerarii with 2 spines, 2–4 auxiliary setae and 20–29 trilocular pores; the 6th cerarii of the cephalothorax with 2 or 3 spines, 4–5 auxiliary setae and 25–33 trilocular pores; the 1st abdominal cerarii with 2 spines, 1–3 auxiliary setae and 25–27 trilocular pores; the 5th and the 6th abdominal cerarii with 2 spines about 0·016 mm. long, 4 auxiliary setae and 28–30 trilocular pores; the 7th abdominal cerarii with 2 spines about 0·020 mm. long, a few auxiliary setae and with 32–35 trilocular pores; the 8th abdominal or anal cerarii with 2 thick spines about 0·025 mm. long, 4 long auxiliary setae and 52–56 trilocular pores, some of the latter forming a compact group around the spines whilst the remainder are rather widely separated; the anal cerarii situated on a small sclerotised area. The body setae of different lengths, some up to 0·024 mm. along a median dorsal line on the thorax and others up to 0·023 mm. on the margin of the anterior dorsal ostioles; the pre-anal setae 0·075–0·080 mm. long.

This species was collected on bananas sent to Leningrad from Colombia on 23rd March, 1939.

Pseudococcus colombianus, sp. n.

Adult Female.—Body oval, pinkish, length 1·8 mm., breadth 0·9 mm.; antennae 7-segmented, length of the segments in microns: I–44; II–52; III–54; IV–40; V–32; VI–38; VII–88; apical segment sometimes incompletely divided into two segments; eyes hemispherical, with 3 discoidal pores near each; hind femora and tibia with some translucent pores; circulus oval, large; the length of the anal ring setae about 0·105 mm. and of the anal lobe setae about 0·135 mm.; the anal lobes ventrally with a small sclerotised area and 4 setae. Single multilocular pores or groups of pores distributed on the cephalothorax and on the 1st and 7th abdominal segments; similar pores arranged in a transverse row on the 2nd and 3rd abdominal segments, and in two transverse rows on the 4th–6th abdominal segments dorsally; ventrally they occur in small groups and two transverse bands on the cephalothorax, and a wide transverse band on the 2nd–8th abdominal segments; irregular discoidal pores occur dorsally only on the posterior abdominal segments; trilocular pores distributed over the whole body; mushroom-shaped ducts with one micropore and two body setae, one duct occurs near the 4th abdominal cerarii; tubular ducts of two sizes, some about 0·005 mm. long and 0·004 mm. thick distributed along the body margin on both surfaces of the body and singly along the median line, others about 0·008 mm. long and 0·003 mm. thick form a transverse row on the abdominal segment ventrally. With 17 pairs of cerarii: the 1st, 2nd and 6th cerarii of the cephalothorax with 3 spines about 0·011 mm. long, 2–3 auxiliary setae and 10–17 trilocular pores; the remaining cerarii of the cephalothorax with 2 spines, 1–3 auxiliary setae and 10-16 trilocular pores; the 1st-6th abdominal cerarii with 2 spines about 0·012 mm. long, 1-3 auxiliary setae and 13–17 trilocular pores; the 7th abdominal cerarii with 2 spines about 0·015 mm. long, 2–3 auxiliary setae and 17–19 trilocular pores; the 8th abdominal or anal cerarii with 1 or 2 thick spines about 0·022 mm. long, 3 auxiliary setae and 37–39 trilocular pores, the latter do not form a group around the spines; anal cerarii surrounded by a sclerotised area. Body setae few in number, of different lengths, some 0·021–0·025 mm. along a median dorsal line on the thorax, others 0·012–0·018 mm. on the margin of the anterior dorsal ostioles; the pre-anal setae about 0·045 mm. long.

This species was collected on bananas sent to Leningrad from Colombia on 23rd March, 1939.

References.

GREEN, E. E. (1921). Observations on the British Coccidae, with descriptions of new species. VI.—Ent. mon. Mag., **57,** pp. 146–152, 189–200, 11 figs.

HOUGH, W. S. (1925). The anal lobe of *Pseudococcus comstocki* Kuw. (Homop. : Coccidae).—Ent. News, **36,** pp. 52–54, 7 figs.

——. (1925a). Biology and control of Comstock's Mealy Bug on the Umbrella Catalpa.—Tech. Bull. Virginia agric. Exp. Sta., no. 29, 27 pp. 7 figs. 5 diagr.

THE FEEDING OF "GAMMEXANE" AND DDT TO BOVINES.

By S. G. Wilson.
Senior Veterinary Research Officer, Entebbe.

A "Gammexane" dispersible powder (P.530) and a DDT emulsion (A.19) have been used for several months in spraying experiments for the control of the various bovine tick species. Preliminary results were disappointing because the lethal effect of both insecticides was lost (from the coat and skin of animals) in from 4 to 6 days. It was not known whether the sprays were being washed off or absorbed, but before they could be used on any considerable scale it was necessary to investigate the effects on body tissues. Cattle feeding experiments were therefore carried out and the results on cattle and on certain species of blood-sucking Arthropods were studied. For these experiments, D.929, powdered benzene hexachloride, containing 13 per cent. of the active gamma isomer and a DDT powder containing 83 per cent. of the para-para isomer were used. The effects of the gamma isomer and DDT in the bovine blood were estimated biologically by feeding both *Rhipicephalus appendiculatus*, Neum., and *Glossina palpalis*, R.-D., on the treated animals. *G. palpalis* proved to be the more suitable as they readily suck blood, the state of engorgement can be immediately assessed and, as will be shown later, the effects of benzene hexachloride can be seen within a very few hours. Ticks take some hours to attach themselves and, if they engorge and live, some weeks must elapse before the results can be definitely assessed. The first step was to ascertain the lethal dose and optimum tolerated dose of D.929.

Preliminary Feeding Tests with Powdered Benzene Hexachloride.

(*a*) Calf No. 5591 weighing 160 lbs. was drenched on two successive days with 36·5 gms. of D.929 which was equivalent to a dose of 0·5 gm./kg. of the powder, or 65 mg./kg. of the active gamma isomer. This rate of dosage proved rapidly toxic; general paralysis and hypersensitivity of the skin and muscular tremors developed and the calf died 90 hours after the first dose.

Active hungry *Glossina palpalis*, 4 to 5 days old, were fed on this calf. On the 2nd day, *i.e.*, 26 hours after the first dose of D.929 and 2 hours after the second dose, five *G. palpalis* were allowed to feed. Two ♂♂ engorged fully, became paralysed within 10 minutes and, unable to fly or walk, lay on their backs with legs and body twitching spasmodically at fairly regular intervals of a few seconds. They died 6 hours later. One ♀, slightly engorged, remained active for about 4 hours, but was paralysed within 6 hours and was dead in 24 hours. One ♂ and 1 ♀ showed no signs of engorgement; the ♂ remained alive for 48 hours before being discarded but the ♀ died in 26 hours. Twenty *G. palpalis* in a second box were allowed to feed at the same time and gave the following results. Five ♂♂ and three ♀♀ became fully engorged and were unable to balance or walk in 10 minutes and died a few hours later. One ♂ and one ♀ were only partially engorged and the onset of paralysis was delayed but they were dead in 20 hours after feeding. One engorged ♀ died in 24 hours but nine unengorged flies (3 ♂♂ and 6 ♀♀) remained alive for 48 hours and were then fed on healthy calves and continued to survive.

On the 3rd day, 50 hours after the first dose of D.929 and 26 hours after the second dose, eleven active flies 6 days old were allowed to feed. Five ♂♂ and one ♀ were fully engorged and 4 hours later one ♂ was dead and the remaining 3 flies were unable to balance or walk and died 6 hours later. One ♂ and four ♀♀ only partially engorged were active 4 hours after feeding, but all were dead in 24 hours.

On the 4th day 6 flies were fed, *i.e.*, 72 hours after the first dose. One ♂ and one ♀, fully engorged, became paralysed in a matter of 5 to 7 minutes, and although still twitching 2 hours later, were dead in 4 hours. Two ♀♀, slightly engorged, were unable to balance or walk in 2 hours and lying on their backs moved around in circles with their wings fluttering and died in 5 hours. The remaining ♂ and ♀ died in 6 hours.

(*b*) A calf No. 5592, weighing 224 lb. was given 25·5 gm. of D.929 on two successive days and 13 gm. on the 3rd and 4th day. This corresponded to 0·25 gm./kg. and 0·125 gm./kg. of the compound and 32·5 gm./kg. and 16·25 gm./kg. of the gamma isomer. The benzene hexachloride powder mixed with water was given by mouth as a drench. *Glossina palpalis* were fed each day from the 2nd to 41st day, and the results were recorded daily. These results were consistently good in that engorged or semi-engorged tsetse were killed within 2 to 24 hours of feeding and a few of the detailed results are given in Table I.

The typical symptoms shown by the affected flies were very similar in all cases. Those fully engorged usually showed signs of paralysis in 10–15 minutes; they soon became unstable and lay on their backs, fluttering their wings, and with their legs and body twitching at fairly regular intervals of a few seconds. In the ♂, the hypopygium was active, the claspers were extruded, and a drop of black or white fluid was expressed. Death occurred on or before the 6th hour after engorging. Engorgement in many flies was gross and exceeded anything seen in control experiments though this was not so noticeable during the later days of the experiment. Many of the semi-engorged flies were easy to classify as, when still alive, the anterior portion of their abdomen was slightly convex and tinged red with blood. Such flies showed very similar symptoms to the fully-engorged individuals but the sequence of events was slower and death occurred between the 6th and 24th hour. In cases where engorgement was doubtful the abdomen was dissected after death for traces of blood. Some non-engorged flies died, but this may have been due to natural causes or to the fact that they engorged too little for blood to be detected in the abdomen. There was little evidence throughout the experiment to show that mere contact with the skin without engorgement of blood was able to kill flies, *i.e.*, little benzene hexachloride was being excreted through the skin.

The benzene hexachloride was excreted very slowly as sufficient concentration remained in the blood to kill tsetse until the 41st day. On that day a fully engorged fly died relatively slowly and semi-engorged flies recovered. Mr. Barlow, Chemist of the Colonial Insecticide Research Team, estimated that on the 36th day 2·1 mgm. of benzene hexachloride were present in 100 cc. of venous blood.

Throughout the whole of the 40 days of the experiment this calf was exposed to ticks infested with East Coast Fever but no adult *R. appendiculatus*, *R. evertsi*, Neum., *Amblyomma variegatum*, F., and *Palpoboophilus decoloratus*, Koch, remained attached long enough to become fully engorged. Engorged nymphs were also absent, but it is difficult to be certain about newly attached nymphs and larvae as, owing to their minute size, they can easily escape detection; at the same time there was no infection of East Coast Fever, although a control calf No. 5606 became infested with ticks and contracted the disease with the normal incubation period.

(*c*) A single dose of 16 gm. of D.929, *i.e.*, at the rate of 0·25 gm./kg., was given to Calf No. 5598. This bovine was grazed from the 3rd to the 17th day in a tick-infested paddock. *G. palpalis* were fed daily for 14 days. This rate of dosage was repeated on three other calves, with very similar results. Benzene hexachloride appeared in the blood from 4 to 8 hours after dosage and remained in the blood in sufficient concentration to kill engorged ticks and tsetse fly until the 10th to 12th day. Flies which engorged on untreated calves showed no similar mortality. This

TABLE I.

G. palpalis fed on Calf 5592.

Days	Dose of D.929 in gm./kg.	Number of flies	Number of flies engorged	Number of flies partially engorged	Number of flies non-engorged
1st	0·25	Nil	Nil	Nil	Nil
2nd	0·25	14	4♂♂ 1♀. Signs of distress 1 hour. Paralysed in 2 hours. Death in 6 hours	2♂♂ 5♀♀. Paralysed 6 hours. 1♂ 2♀♀ still twitching 22 hours. All dead 24th hour	1♂ 1♀. Died in 17 hours
3rd	0·125	12	4♂♂ powerless 2 hours. Died 6 hours	1♂ paralysed still twitching 24 hours. Died 26 hours	1♀ died. 6 flies active 48 hours
4th	0·125	20	6♂♂ 3♀♀. Signs of paralysis in 10 minutes. Paralysed 2 hours. Died 5 hours	1♂ 1♀. Died 5th hour. 1♂ 3♀♀ died 7th hour	5 flies active 24 hours and fed clean animal
7th	Nil	13	7♂♂ 3♀♀. Paralysed or dying in 2 hours	1♀ able to fly 3rd hour. Died 6th hour	2 flies active 48 hours and re-fed clean animal
10th	Nil	11	1♂ 6♀♀ still twitching 3rd hour. Died 6th–10th hour		1♂ 1♀ died 24 hours. 2♂♂ alive 24 hours
12th	Nil	13	2♂♂ paralysed 1 hour. Died 2 hours. 1♂ died 3 hours. 1♂ active 3 hours, died 4 hours	2♂♂ 2♀♀ paralysed 4 hours and died 6th hour. 2♀♀ slight paralysis 8th hour, died 12 hours	3 flies active 36 hours and re-fed
19th	Nil	8	1♂ 2♀♀ dead 2 hours	3♀♀ fully paralysed 4 hours. Dead 6 hours	1♂ 1♀ alive 4 hours
24th	Nil	7	1♂ 2♀♀ still twitching 12 hours. Dead 15 hours	2♀♀ dead 24 hours	1♂ alive 48 hours. 1♀ dead 48 hours
29th	Nil	10	3♂♂ dead 24 hours	1♂ fully paralysed 24 hours—1♂ dead 24 hours	3♂♂ 2♀♀ alive 48 hours
33rd	Nil	13	3♂♂ 1 ♀ dead 3 hours	4♂♂ 2♀♀ dead 24 hours	1♂ 2♀♀ alive 48 hours
36th	Nil	9	2♂♂ paralysed 7 hours. 2♂♂ 1♀ dead 7 hours	2♂♂ 2♀♀ dead 24 hours	—
40th	Nil	7	2♂ 1♀ dead 24 hours	3♂♂ 1♀ dead 4–6 hours	1♂ alive 48 hours
41st	Nil	5	1♂ slightly paralysed 3 hours. Paralysed 4 hours, dead 8 hours	2♂♂ 2♀♀ all recovered	—

dosage appeared to have little toxic effect on bovines ; two of the calves showed some restlessness and hypersensitivity about the 4th hour after administration, but this soon passed off. The appetite was unimpaired and there was no loss of weight nor obvious blood changes. The results of feeding *G. palpalis* on Calf No. 5598 are given in Table II.

TABLE II.

G. palpalis fed on Calf B. 5598.

Days of experiment	Number of flies fed	Number of flies fully engorged	Number of flies partially engorged	Number of flies non-engorged
1st	16	2♂♂ 4♀♀ paralysed and dying in 2 hours	1♂ 5♀♀ dead or dying in 2 hours	1♂ 3♀♀ active 48 hours
2nd	13	4♂♂ 1♀ dying 3 hours	3♀♀ dead 24 hours	3♂♂ 2♀♀ alive 24 hours and re-fed
3rd	15	4♂♂ 3♀♀ dying 3 hours	1♂ 1♀ dead 24 hours	4♂♂ 2♀♀ alive 24 hours and re-fed
4th	17	3♂♂ 2♀♀ dying 4 hours. 1♂ still attempting to fly 4 hours, dead 8 hours	1♀ dead 24 hours	1♂ dead 24 hours. 3♂♂ 6♀♀ alive 24 hours and re-fed
5th	14	3♂♂ 2♀♀ fully paralysed 4 hours. 2♂♂ dying 6 hours	1♂ 3♀♀ dying 24 hours	2♂♂ 1♀ alive 24 hours and discarded
6th	9	4♂♂ dying 2 hours	1♂ 1♀ dying 6 hours	1♂ 2♀♀ active 24 hours and re-fed
8th	9	1♂ 2♀♀ dead 4 hours	3♂♂ 2♀♀ dead 24 hours	1♂ alive 48 hours
10th	8	1♀ dead 3 hours	2♂♂ 1♀ dead 15 hours	2♂♂ 1♀ dead 48 hours. 1♂ alive
12th	9	1♂ affected in 2 hours dead 12 hours	1♀ dead 12 hours	3♂♂ 4♀♀ alive 24 hours and re-fed
13th	6	—	1♂ dead 24 hours	2♂♂ 3♀♀ alive 48 hours
14th	16	8 fully engorged. Some appeared slightly affected 3rd to 9th hour but *all lived*	3 semi-engorged not affected and re-fed 24 hours	5 no apparent engorgement. All alive 24 hours and re-fed

The effect of this single dose of benzene hexachloride on tick control was not so encouraging. From the 3rd to the 17th day after treatment the calf was grazed in a tick infested paddock, the ticks being infected with *Theileria parva*, and careful examination showed that it remained free of adult ticks and fully engorged nymphs until the 10th day. A few newly attached *R. appendiculatus* nymphs were observed daily but these invariably died without engorging. On the 10th day live *R. appendiculatus* and *A. variegatum* nymphs were found attached, and these were collected fully engorged on the 14th day. The *A. variegatum* nymphs died without moulting, but two *R. appendiculatus* nymphs moulted to adults in 23 days. It is obvious from the temperature chart given below that some of the *R. appendiculatus* nymphs remained on long enough during the early days of exposure to infect the calf with East Coast Fever.

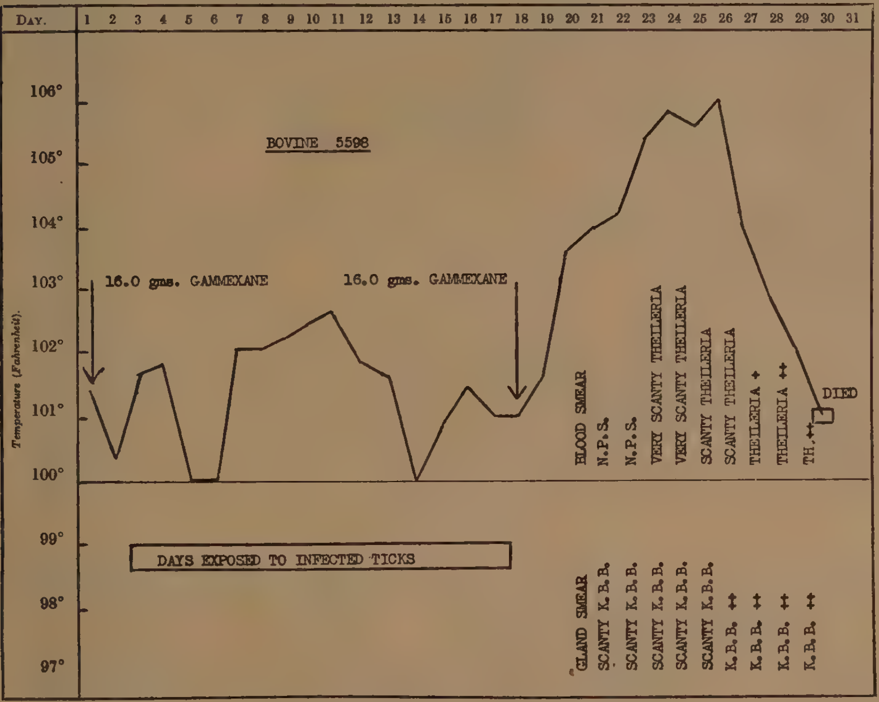


Fig. 1.—Temperature chart of Bovine 5598 showing course of East Coast Fever after dosage with benzene hexachloride.

K.B.B. = Koch's Blue Bodies; N.P.S. = No parasites seen.

The first signs of East Coast Fever were seen on the 19th day. As the usual incubation period of virulent infections of East Coast Fever in this paddock is 13 days, the ticks must therefore have infected this animal on or before the 6th day after dosage. A single dose of D.929 at the rate of 0·25 gm./kg., therefore, gives very short protection against East Coast Fever.

Feeding Benzene Hexachloride at varying Intervals.

From the first series of experiments, it appeared that 0·25 gm./kg. was a reasonably safe dosage as none of the animals showed serious toxic symptoms, but the protection from East Coast Fever was of short duration. Experiments were therefore designed to ascertain whether, if this or a lower dosage could be safely repeated at varying intervals, any increased protection could be conferred.

Six calves, susceptible to East Coast Fever, were grazed with native cattle for 28 days on pastures where tick species, *Rhipicephalus appendiculatus*, *R. evertsi*, *R. simus*, Koch, *Amblyomma variegatum* and *Palpoboophilus decoloratus* were known to be prevalent. East Coast Fever was known to occur in the area. Five of the six calves were given D.929 and the sixth acted as a control. Tick counts were made on two occasions each week, and tsetse were fed daily after the last dose had been given.

(a) *0·25 gm./kg. repeated four times at every 7th day.*

Calf No. 5568, weighing 204 lb., was given 23·15 gm. of D.929 every 7th day for a month. The number of live ticks remaining attached was negligible throughout the 28 days, though numerous dead ticks were collected, and the calf did not contract East Coast Fever. *G. palpalis* were killed on the 20th day after the fourth and final dose, but not on the 22nd and 24th days. There was therefore some cumulative effect resulting from the four doses as compared with a single dose. The animal weighed 202 lb. on the 28th day and there was therefore some loss of weight due probably to the benzene hexachloride and the poor grazing.

(b) *0.25 gm./kg. repeated twice at 14-day intervals.*

Calf No. 5571 weighing 236 lb., was given 26·83 gm. of D.929 on the 1st and 14th day, and ticks were counted twice weekly for a month with the following results :—

TABLE III.

Tick counts from Calf No. 5571 receiving 0·25 gm./kg. D.929 on 1st and 14th days.

Days of experiment	1st	3rd	7th	10th	14th	17th	21st	24th	28th
Dosage of D.929 ...	26·8 gms.	—	—	—	26·8 gms.	—	—	—	—
Number of live ticks attached	—	3	3	7	17	nil	1	3	5

The cumulative effect of the drug is again shown as results during the second 14 days were better than during the first fortnight.

On the 14th day 3 partially engorged *R. appendiculatus* ♀♀ were found dead on the ears, and many dead adults and nymphs of various species were collected from the body. In addition 14 live ticks at various stages of engorgement were counted. A second dose of D.929 was given and ear bags attached collected all the ear ticks dead in 24 hours. On the 28th day 3 ♂♂ and 1 ♀ *R. appendiculatus* were attached to the ears and 1 ♂ on the eye, but none of these was fully engorged. The protection from East Coast Fever under this dosage was not complete, for on the 42nd day of the experiment, or 14 days after they had been removed from exposure to ticks, symptoms of East Coast Fever developed.

Feeding *G. palpalis* were being killed on the 15th day, but not on the 17th day after the second dose.

(c) *0·125 gm./kg. repeated four times at every 7th day.*

Calf No. 5577, weighing 195 lb., was dosed with 11·25 gm. of D.929 every 7th day for 28 days. There was excellent tick control as the number of live ticks attached on any one day on the ears never exceeded 7 compared with an average of 32 on two control cattle, and these all died within 48 hours of attachment and failed to engorge. Some days after the 4th and final dose, 2 ♂♂ and 1 ♀ *R. appendiculatus* were attached to the left ear, and when a feeding bag was placed on the ear the ticks were collected dead two days later, *G. palpalis* were also fed on three occasions with the results shown in Table IV.

The benzene hexachloride showed signs of disappearing on the 10th day, and fully engorged flies recovered on the 14th day. The animal lost 5 lb. in weight during the experiment.

TABLE IV.

G. palpalis fed on Calf No. 5577.

Day after receiving final dose of D. 929	Number of flies used	Number of flies fully engorged	Number of flies semi-engorged	Number of flies not engorged
8th	7	1♂ 3♀♀ dead 2 hours	1♂ 2♀♀ dead 4 hours	—
10th	3	1♂ paralysed 3 hours, still twitching 24 hours, dead 3 hours	1♂ 1♀ active 48 hours	—
14th	10	2♂♂ 1♀ recovered	1♂ paralysed 24 hours. 1♂ 2♀♀ alive 48 hours	1♂ dead 24 hours. 2♂♂ alive 48 hours

(d) *0·125 gm./kg. of D.929 given twice weekly.*

Calf No. 5574, weighing 198 lb., was given 11·3 gm. of D.929 on the 1st and 4th day of each week for 4 weeks, and exposed to tick infestation with native cattle. After the 3rd dose there was almost complete tick control as only one or two very inactive ticks were found on any one day. Tsetse were fed on 13 occasions, and some of the results are given in Table V.

TABLE V.

G. palpalis fed on Calf No. 5574.

Day after receiving final dose of D.929	Number of flies used	Number of flies fully engorged	Number of flies semi-engorged	Number of flies not engorged
5th	5	1♀ dead 2 hours. 1♀ dead 4 hours	2♂♂ dying 4 hours	1♂ alive 48 hours
11th	6	4♂♂ paralysed 1 hour, dying 4 hours	—	1♂ 1♀ alive 48 hours
14th	5	1♀ fully paralysed 3 hours; dead 4 hours	2♀♀ 1♂ dying 3 hours	1♂ alive 48 hours
15th	7	2♀♀ dead 4 hours	1♂ 1♀ twitching 24 hours, died	1♂ 2♀♀ alive 48 hours
18th	9	3♂♂ 1♀ dead or dying 2 hours	2♀♀ dead 24 hours	2♀♀ alive 48 hours. 1♀ died 48 hours
21st	12	2♂♂ 3♀♀ dead 3 hours	1♂ 1♀ dead 24 hours	2♂♂ 3♀♀ alive 48 hours
23rd	11	1♂ still twitching 9 hours, dead 12 hours	2♂♂ 2♀♀ twitching 9 hours, dead 12 hours. 1♂ 1♀ dead 9 hours. 1♀ dead 24 hours	1♂ dead 36 hours. 2♀♀ alive 48 hours
29th	5	—	1♂ 2♀♀ dead 24 hours. 1♀ still twitching 24 hours, dead 30 hours	1♂ active 48 hours

The results with this calf were similar to those in the case of Calf No. 5568, but by giving 0·125 gm./kg. twice weekly the cumulative effect was more marked than at 0·25 gm./kg. once every week. The animal lost 10 lb. in weight during the 28 days of the experiment.

(e) *0·1 gm./kg. given weekly.*

Calf No. 5579, weighing 206 lb., received 9·4 gm. of D.929 weekly for 4 weeks. The tick counts are given in Table VI.

TABLE VI.
Tick counts on Calf No. 5579.

Day of experiment	1st	3rd	7th	10th	14th	17th	21st	24th	28th
Dosage of D.929 in gms. ...	9·4	—	9·4	—	9·4	—	9·4	—	—
Number of adult live ticks attached	—	2	10	3	13	1	2	4	6

More complete tick control was achieved after the 3rd dose on the 14th day than during the preceding days. On the 28th day of the experiment 3 ♂♂ and 3 ♀♀ *R. appendiculatus* were partially engorged, but they dropped off next day and only survived for a few days and failed to lay. The calf, however, developed East Coast Fever on the 40th day of the experiment or 12 days after it had been removed from exposure to ticks. Engorged and semi-engorged *G. palpalis* died on the 11th day but survived on the 13th day after the 4th and final dose.

(f) The control Calf No. 5580 developed East Coast Fever within 23 days after being herded with native cattle and subjected to tick infestation.

Effect of Benzene Hexachloride on the Course of East Coast Fever.

Calf 5606 was herded in the East Coast Fever paddock together with Calf 5592 to

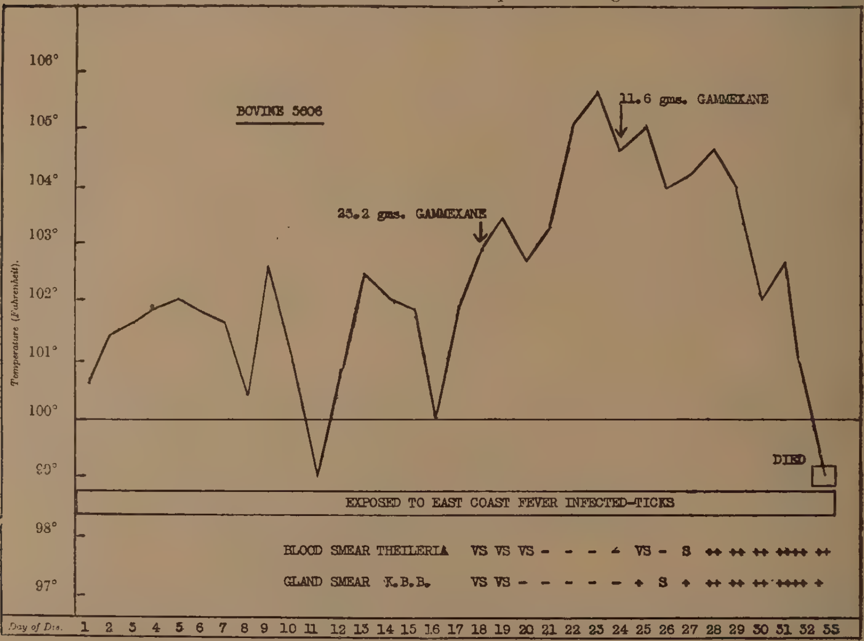


Fig. 2.—Temperature chart of Bovine 5606 showing course of East Coast Fever infection after treatment with benzene hexachloride.
K.B.B.=Koch's Blue Bodies ; VS=Very scanty ; S=Scanty.

act as a control to the latter which had been dosed with D.929 in the preliminary feeding tests. Calf 5592 did not contract East Coast Fever, but Calf 5606 showed the usual initial symptoms on the 18th day with temperature of 102·8°F., small piroplasms in the red blood cells and scanty Koch's Bodies in the gland smears. On the same day it received 23·2 gm. of D.929 which was equivalent to 0·25 gm./kg. Eighteen hours later, 2 ♂♂, 1 ♀ and 7 nymphs of *R. appendiculatus* and 1 ♂, 1♀, 62 nymphs and 34 larvae of *A. variegatum* species were collected dead from various parts of the coat. No living ticks were found. A second dose of 11·6 gm. or 0·125 gm./kg. was given on the 24th day, but the calf died of East Coast Fever on the 33rd day. As will be seen from the temperature chart below, the benzene hexachloride had no effect on the temperature reaction or course of the disease.

The initial dose on the 18th day may have had some action on the production of Koch's Bodies, as blood and gland smears were negative from the 20th to 23rd days, but from the 24th day onwards, Koch's Bodies re-appeared and were numerous until death. This supports the conclusion reached in the case of Calf 5598, which died of East Coast Fever in spite of benzene hexachloride treatment.

Effect of Benzene Hexachloride on the Development of *Trypanosoma congolense* Infections.

Two calves, Nos. 5561 and 5586, each received D.929 as follows: 1st day 0·25 gm./kg.; 2nd and 3rd day 0·125 gm./kg.; and 7th day 0·125 gm./kg.

On the 2nd day these two calves, together with a control Calf No. 5596, were sub-inoculated with 0·5 cc. citrated blood infected with a known pathogenic strain of

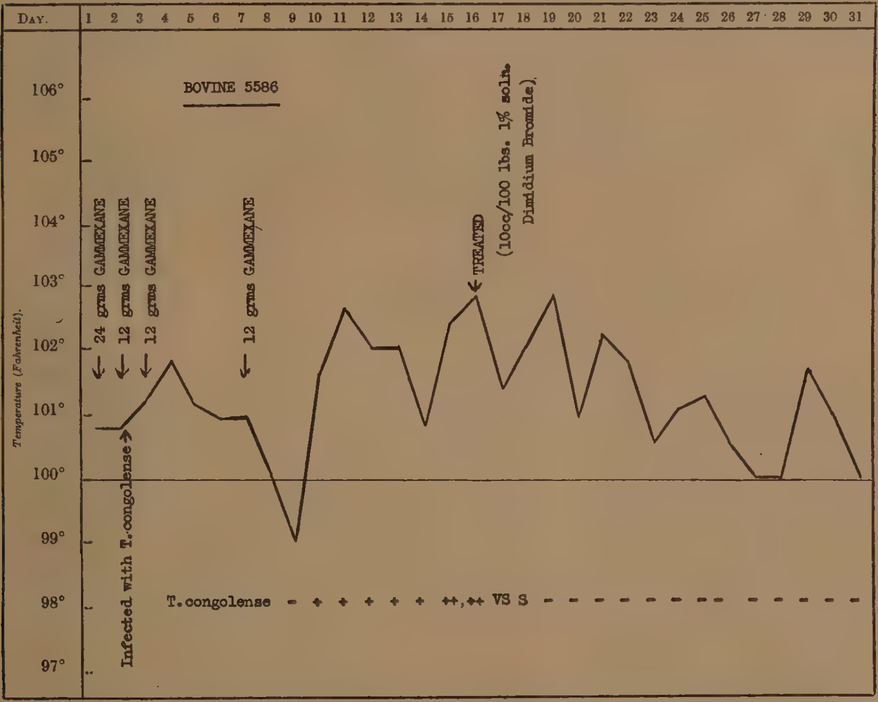


Fig. 3.—Temperature chart of Bovine 5586 showing course of *T. congolense* infection after treatment with benzene hexachloride.
VS=Very scanty; S=Scanty.

T. congolense. This dosage had no effect whatever on the development of the trypanosomes as they appeared in the blood of the treated animals at the same time as in the control calf. The course of the infection in Calf No. 5586, one of the calves receiving treatment, is shown in fig. 3.

Further Feeding Tests with Benzene Hexachloride.

It was obvious from the preliminary feeding test that a dose of 0·5 gm./kg. was toxic, but 0·25 gm./kg. is tolerated when given on two successive days or repeated at weekly intervals. The effects of doses between 0·25 gm./kg. and 0·5 gm./kg. were still undetermined. Seven bovines were used in this experiment and during the first seven days they were grazed in an East Coast Fever tick-infested paddock. A summary of the results is given in Table VII.

TABLE VII.

Toxicity tests with single dose of D.929.

Expt. No. of Bovine	Age in months	Weight in lbs.	Rate of dosage	Total D.929 given in gm.	Total dose in gm. of gamma isomer	Result
5433	27	392	0·4 gm./kg.	71·3	9·3	Condition remained good
5451	24	416	Do.	75·6	9·8	Paralysis in 18 hours. Good recovery
5414	29	476	0·35 gm./kg.	75·7	9·8	Condition remained good
5617	8	180	Do.	28·6	3·7	Dead 18 hours
5415	30	440	0·3 gm./kg.	60·0	7·8	Condition remained good
5570	25	328	Do.	44·7	5·8	Died 6th day
5485	19	250	Control	—	—	Developed East Coast Fever on 13th day

At 0·4 mg./kg., Bovine 5433 showed no clinical signs whatever, but the benzene hexachloride only remained in the blood for 11 days in sufficient concentration to kill *G. palpalis*. Bovine 5451 showed signs of lumbar paralysis in 12 hours, and by the 24th hour the animal was unable to rise. On the 3rd day there was considerable improvement and the animal was attempting to walk, but there was still muscular tremors, and the flexar tendons of the tarsus appeared contracted causing "knuckling" of the metacarpo-phalangeal joints so that the animal walked on its toes. This latter symptom passed off in four days, and recovery was then uneventful.

At 0·35 gm./kg., Bovine No. 5617, a young animal weighing 180 lb., died within 18 hours. The larger animal No. 5414 remained in good condition without showing any toxic symptoms. Engorged *G. palpalis* were killed on the 13th day, but not on the 16th day after dosage.

Very similar results were observed with doses of 0·3 gm./kg. The larger bovine, No. 5415, remained in good condition and urine analysis was normal. The smaller bovine, No. 5570, showed restlessness, staring eyes, increased respiratory rate and tremors of the shoulder muscles on handling in 12 hours, and movements soon became uncoordinated and the beast was unable to rise in 24 hours. The animal appeared brighter on the 3rd day, but death occurred on the 6th day without the beast ever regaining power to rise or walk. Gross post-mortem changes were seen in the brain, liver, kidneys and bladder. The brain substance was very firm on section, and the meningeal vessels were very congested. The liver was very enlarged, friable and congested and microscopically showed small irregular areas of focal necrosis within each lobule. The gall-bladder was fully distended with dark green bile. The kidneys were normal in size, but the cortex and medulla were dark-brown and congested, and there was a focal glomerulo-nephritis. The affected glomerule showed increased cellularity and congestion with the cells lining the capsule of Bowman loosened or desquamated. The tubules, especially the proximal convoluted tubules, were also affected. The bladder was enormously distended, and showed signs of congestion with partial or even complete shedding of the superficial epithelial layers over wide areas. There were no very marked changes in the alimentary mucosa.

The control animal, No. 5485, developed East Coast Fever on the 13th day after exposure in the tick-infested paddock, but all the surviving treated animals were protected.

Feeding DDT to Bovines.

A DDT powder containing 83 per cent. of the para-para isomer was fed to two bovines, *i.e.*, Bovine No. 5519 was drenched with 65 gm. of the powder at the rate of 0·5 gm./kg.; and Bovine No. 5572 was drenched with 19·5 gm. at the rate of 0·25 gm./kg. They showed no apparent ill effect. Eighteen *Glossina palpalis* were allowed to feed on each animal on the 18th hour after dosage and ten were obviously engorged. Two fully engorged ♂♂ became paralysed after 3 hours, and died in 8 hours, and 2 ♂♂ and 1 ♀ were found dead after 24 hours, but all the remaining flies were active. Tsetse fed on the 24th and 48th hour after dosage were not affected.

Apart from showing that DDT can safely be ingested by bovines in relatively large doses this experiment had little practical value.

Summary.

Benzene hexachloride and DDT are relatively new insecticides and their veterinary value when administered orally to bovines has not yet been fully investigated.

The first series of experiments showed that 0·5 gm./kg. on two successive days of D. 929, was toxic; 0·25 gm./kg. on two successive days followed by 0·125 gm./kg. on the 3rd and 4th days was well tolerated, and the benzene hexachloride remained in the blood in sufficient concentration to kill feeding tsetse and ticks up to the 41st day. After a single dose of 0·25 gm./kg. the benzene hexachloride remained in the blood for only 10 to 12 days, and failed to protect the animal from East Coast Fever when it was grazed on heavily infested pasture.

Five calves susceptible to East Coast Fever were dosed with D.929 and exposed to attack by the common tick species under African farming conditions. A dose of 0·25 gm./kg. given weekly protected a calf from East Coast Fever, but there was no protection when the same dose was given once a fortnight. A dose of 0·125 gm./kg. given weekly also protected, whereas 0·1 gm./kg. failed. At all these dosages the concentration of benzene hexachloride in the blood was sufficient to kill feeding *G. palpalis* during the period the calves were under treatment and for periods of 10 to 25 days after the final dose.

D.929 given to animals suffering from East Coast Fever and Trypanosomiasis had no effect on the course of these diseases.

Doses of 0·3 gm./kg. (39 mg./kg. gamma isomer) are toxic to young animals but older animals showed a greater tolerance. Hypersensitivity, muscular tremors, and general paralysis were the main toxic symptoms, and gross pathologicaal changes were most obvious in the liver and urinary system. The median lethal dose of benzene hexachloride given orally for calves is therefore lower than for small laboratory animals as recorded by Cameron (1945). Only crude benzene hexachloride powder (13 per cent. gamma) was used in the present experiments, but Slade (1945) has shown that this is markedly less toxic than the purified gamma powder.

Little experimental work was done with DDT as the results with benzene hexachloride were more promising both in the control of ticks and tsetse.

Acknowledgements.

My grateful thanks are due to the members of the Colonial Insecticide Research Team whose willing co-operation in the supply of materials made this work possible, to Mr. S. G. Laws, F.I.M.L.T., of this laboratory, for valuable assistance in its preparation for publication and to the Director of Veterinary Services for permission to publish this paper.

References.

CAMERON G. R. (1945). Risks to man and animals from the use of DDT.—Brit. Med. Bull., **3,** pp. 783–785.

SLADE, R. E. (1945). Chem. & Industr., **64,** pp. 314–319. (Quoted by H. Kirk (1946).—Vet. Rec., **58,** pp. 465–466.)

SOME DIFFICULTIES IN DETERMINING BY MEANS OF FIELD SAMPLES THE TRUE VALUE OF PARASITIC CONTROL.

By F. J. Simmonds, M.A., B.Sc., Ph.D.
Commonwealth Bureau of Biological Control.

E.M.I

Investigations on the possible biological control of a particular insect pest usually have as their object the discovery of parasitic or predacious species which, when liberated in a different area without their own natural enemies, will in time bring about an economic reduction in the numbers of the pest concerned. Hence it is desirable to have some standard of comparison between various parasites attacking a pest of their efficiency as controlling agents. The standard normally used is that of the percentage of hosts in a certain stage found to be attacked by a given parasite species. Samples of host material are taken in the field and either dissected or reared in the laboratory. In either case the parasite species present are noted, the percentage of the hosts parasitised by each species worked out (together with the figures for hyperparasitism, if any) and a comparison made between them as to their relative value as controlling agents. Often such estimations of the value of a parasite species are based on a few small localised collections of field material ; but apart from any criticism arising from a statistical consideration of the methods of sampling there are certain difficulties of a biological nature inherent in the estimation of the degree of control exerted by individual parasite species, Some of these will be outlined below.

Removal of Field Material from further possible Attack.

One defect of any sample made to determine the extent of parasitism is quite obvious. When material is taken from the field into the laboratory it is removed from any further attack by parasites. This may give an entirely wrong impression of the relative importance of different parasite species. Supposing, for example, that a number of nearly full-grown host larvae are collected. A parasite species that attacks small or medium-sized larvae will have adequate representation in the sample, unless the hosts are prevented from attaining full size by the presence of the developing parasite, as often occurs, or unless the species involved emerges from the host before the latter is full-grown. A parasite species, however, which attacks full-grown larvae will probably not be represented to the same extent as it would have been had the material remained in the field and been liable to further attack. Often, too, it is impossible to estimate what this further parasitism would be.

Fluctuation of Host and Parasite Populations occurring at the Time of Sampling.

If populations of both host and parasites in the field remained constant, then a single sample which had none of the other defects outlined here would give an adequate idea of the actual degree of parasitism attained by the individual species. But even if all other objections were overcome, there still remains one due to the variation with time in numbers of host and parasites. This not only renders a single sample inadequate to express parasitic activity completely, but also affects the estimates obtained from such a single sample. Supposing that the population of a host remains constant (with respect to the actual stage of the host attacked by the parasite), then, when the emergence of a parasite generation commences, parasitism will increase steadily day by day as oviposition by the parasite proceeds. When emergence of parasite progeny commences (possibly after all oviposition has ceased) the apparent parasitism then declines. Thus, dependent on the point in this cycle at which a sample is taken, the estimate of the degree of parasitism attained will vary, even though the numbers of hosts attacked daily by the parasites may have been constant. Coupled with the factor next to be described it would be possible in this case to get any value of parasitism from zero to 100 per cent. even though parasite activity remained constant. Again, if the host population is fluctuating more complications are introduced, and it is only by a series of samples taken at intervals over an extended period that an adequate estimate can be obtained of the actual degree of control that is being exerted by an individual parasite species. This point was well illustrated by results obtained during a study of parasitism by the frit-fly, *Oscinella frit* (L.), in North America during 1941–1944 when the variations in the population of a pupal parasite, *Spalangia drosophilae*, Ashm. (Spalangiidae, Hymenoptera) were studied in detail. Here the inadequacy of a single or even several samples of field material in obtaining an estimate of parasite activity was clearly indicated, and will be discussed more fully elsewhere. Overlapping generations of both parasite and host add to the complexity, and this general aspect of the problem is closely connected with that which follows in the next sections.

Variation in Duration of a given Stage of the Host due to Parasitism.

The particular defect of field samples becomes very apparent in the consideration of individual cases, as the following example will show. Whilst studying the degree of parasitism of the eggs of *Physonota alutacea*, Boh. (Cassididae, Coleoptera) by *Horismenus* n. sp. (Eulophidae, Hymenoptera) during an investigation of the possible biological control in Mauritius of the weed, *Cordia macrostachya* (Jacq.) R. & S., it was found that in Trinidad very heavy parasitism occurred. Field collections of beetle egg-masses were made, brought into the laboratory for emergence, and an estimate made of the degree of parasitism from the relative numbers of host larvae and parasites. It soon became clear, however, that in addition to the two difficulties mentioned above a third factor was operating to render misleading any estimate of parasitism. Host eggs when parasitised remain as such without producing adult parasites for a far longer period than would elapse if they were unparasitised, and produced host larvae. This delay in emergence following parasitism results in an accumulation of parasitised hosts, and consequently percentages of parasitism obtained from field-collected material tend on this account to be too high. Eggs, however, are only liable to parasitism when they are a few days old and this tends to reduce the apparent degree of parasitism. A correction may be applied to such data to offset both these factors and also the reduction of " true " parasitism by removal of field material from further possible attack when it is brought into the laboratory. One has to consider :—

(*a*) The duration of the egg stage when unparasitised.

(*b*) The age of the egg when attacked by the parasite.

(*c*) The duration of the egg stage when parasitised.

In order to calculate this correction it is necessary to assume that for some period prior to sampling the population of adult beetles and factors governing oviposition remain constant, as does parasite population and activity (see above). That is, a constant number N eggs are laid daily in the population under consideration and of those N eggs p per cent. are parasitised. Also let the duration of the egg stage when unparasitised be a days, and let the developmental time of the parasite be b days. Also let c days be the age of the egg when parasitism usually occurs. Then, ignoring older eggs, consider what happens to eggs laid only on a given day and subsequently :—

No parasitism occurs until *c* days have elapsed when N eggs become suitable for attack.

After *a* days unparasitised hosts begin to emerge, and since parasite activity is assumed to be constant, emergents will be exactly replaced daily by subsequently unparasitised eggs, and the numbers of unparasitised eggs present will then remain constant.

From the b-cth day the parasite and host emergents remain constant, and together equal the number of eggs laid, so that the population remains stable.

This may be summarised as follows :—

Day ...	O	1	2	.C	a	b+c
Eggs laid...	N	N	N	N	N	N
Total eggs present	N	2N	3N	$(c+1)N$	$(a+1)N-\frac{(100-p)N}{100}$	$(b+c+1)N-(b+c+1-a)\frac{(100-p)N}{100}-\frac{pN}{100}$
Parasitised	—	—	—	$\frac{pN}{100}$	$\frac{(a-c)pN}{100}$	$\frac{bpN}{100}$
Unparasitised ...	N	2N	3N	$cN+\frac{(100-p)N}{100}$	$cN+(a-c)\frac{(100-p)N}{100}$	$cN+(a-c)\frac{(100-p)N}{100}$
Emergent parasites	—	—	—	—	—	$\frac{pN}{100}$
Emergent hosts	—	—	—	—	$\frac{(100-p)N}{100}$	$\frac{(100-p)N}{100}$

Let a sample now be taken which gives an observed figure for percentage parasitism of P.

$$\text{Then } P=\frac{\frac{bpN}{100}}{\frac{bpN}{100}+CN+(a-c)\,\frac{(100-p)N}{100}}\times 100$$

$$=\frac{bp}{bp+100c+(a-c)\,(100-p)}\times 100$$

$$=\frac{100bp}{bp+100a+pc-ap}=\frac{100bp}{100a+p\,(b+c-a)}$$

If eggs are parasitised on the same day as laid then c=0 and the correction becomes

$$P=\frac{bp}{bp+a\,(100-p)}+100$$

Now consider an actual sample of *Physonota* eggs taken in the field. In September 1946 in Trinidad unparasitised eggs hatched in approximately 8 days. Eggs were attacked on an average when 3 days old, and development time of the parasite before emergence averaged 15 days approximately.

i.e., a=8
b=15
c=3

From a collection of egg masses made in the field (5th Sept., 1946) a parasitism of 79·7 per cent. (496 parasites, 126 host larvae) was obtained. Masses that had already produced some emergents were discarded.

The true parasitism p is given by :—

$$79{\cdot}7=\frac{15p\times100}{15p+300+5(100-p)}$$

$$=\frac{1500p}{10p+800}$$

$$\therefore 797p+63760=1500p$$

$$63760=703p$$

$$p=90{\cdot}7\text{ per cent.}$$

Thus in this case with *Physonota* the actual true egg parasitism as indicated by this correction is 11·0 per cent. higher than would appear from field data.

The relationship between the true value and observed value for parasitism depends on the relative influences of the two conflicting factors : (1) Age of the stage when the host becomes liable to parasite attack ; the greater this is the more unparasitised (and as yet unsuitable) individuals of the stage under consideration there will be in any collection made, and the figure obtained for parasitism will be lower than the true value of parasite destruction. (2) Prolongation of the duration of a given stage by parasitism ; the longer this is the higher will the apparent parasitism become, giving an abnormally high impression of parasite value in destroying hosts.

This correction may be applied not only to instances of egg samples but, with suitable modifications, to any sample taken in the field to determine the extent of parasitism of a certain stage. It should, however, be borne in mind that the assumptions made at the outset of the calculation as to constancy of environmental conditions, stereotyped behaviour of both host and parasite, and to lack of fluctuations in the populations of the host and parasite over even short periods, render rigid application to natural conditions of very doubtful value.

Several separate Attacks by a parasite Species on the same Host Material.

Particularly in a consideration of the parasitism of overwintering material, instances occur where a parasite species is able to complete more than one generation on a single generation of the host. Thus a sample taken for parasitism will only give a partial estimate of the actual numbers of that particular generation of the host destroyed by the parasite. For example, in North America the sugar-beet webworm, *Loxostege sticticalis*, L., is attacked when a full-grown larva within the cocoon by an ectoparasite, *Cryptus inornatus*, Pratt. In the late summer and autumn adult parasites are active attacking host larvae within their cocoons in the soil. Some of the progeny of these may emerge the same season if the weather is warm, the emergent females making further attacks on the hosts, which are all in diapause and remain so throughout the autumn and winter. Later the subsequent progeny of all these females remain overwintering, some in a state of slowed up development due to low temperature, some in a state of true diapause. In the following spring the former emerge quickly as soon as the weather becomes suitable for further development. Those parasite larvae in diapause only continue development later in the spring when diapause is broken. All these spring-emerging adults, however, attack larvae of the same generation of host as that from which they themselves emerged, since the diapause of the host is not broken until somewhat later in the spring. Thus a sample of host material taken in the autumn will only give indications of a part of the full attack by *Cryptus* and to obtain a better estimate of the numbers of hosts destroyed a sample of cocoons should be taken late in the spring before emergence of the moths, the numbers of both living and empty *Cryptus* cocoons counted, and an estimate of parasitism calculated from this. Similar multi-attack on a generation of overwintering cocoons occurs with the Ichneumonids, *Cryptus sexannulatus*, Grav., and *Ephialtes caudatus*, Ratz., parasites of the codling moth, *Cydia pomonella*, L., in the

South of France. It is obvious in these cases that the destruction caused by such a parasite must not be dismissed as negligible before due consideration has been given to the number of attacks on the same host material of which the parasite is capable.

Differing Availability of Hosts.

This is an aspect of insect parasitism that has been discussed before in connection with the mathematical treatment of the fluctuation of host and parasite populations. If a number of hosts remain in situations (*e.g.*, within fruit, below the surface in stored products, etc.) where they are protected by their position from parasite attack, they will obviously form a nucleus for fresh increases in the population of a pest, however efficient parasites or predators might be in destroying the available hosts. This effect must be taken into consideration when making an estimate of the effectiveness of parasitic control.

Along with the differences in spatial availability of certain host species to ovipositing parasites one must also consider differences in " availability " and in subsequent degrees of parasitism due to discontinuous distribution of host material coupled with the power of the ovipositing parasite to locate the hosts. This is particularly noticeable with certain species of hosts the eggs of which are laid in batches or the subsequent stages of which are gregarious. One often finds that one hundred per cent. parasitism (or nearly so) occurs with individual batches of hosts whereas other batches are unattacked. Light parasitism under such conditions is in many cases rare. For example, with the beetle *Physonota* mentioned above few egg masses escape heavy parasitism but those that do so are completely unparasitised. With the beetle *Schematiza cordiae*, Barber, found on *Cordia* in Trinidad the larvae are gregarious and are attacked by a Tachinid, *Chaetonodexodes marshalli*, Tns., when very small. Parasitism on the whole is high, but there is considerable variation between different " aggregates " of host larvae.

Hence to avoid misleading results care must be taken to secure samples of host material in the field with due consideration to the habits of both host and parasite. This point in sampling is not simply one of size of samples but one of the necessity of ensuring that samples taken really represent all the situations occurring in the host parasite relationship.

It is not claimed that the defects outlined above are the only ones inherent in the method of sampling field populations of insects to obtain an estimate of controlling action of parasites. Nor is it claimed that in no instance can a valid estimate of parasite activity be obtained by a simple sampling method. The object of this note is merely to stress the fact that values obtained in this way should be viewed with caution, and that no estimate of the controlling value of any individual parasite species should be based on the results from a few such samples. In order to gain some true idea of the value of a parasite species as a controlling agent it is necessary to study it in the field over a range of conditions, when its effectiveness may usually be expressed far better in general terms than by a series of " percentages of parasitism ", although these latter may be of use in conjunction with such a general description.

As with attempts at the mathematical prediction of fluctuation of insect populations, narrowly changing environmental conditions may have such a marked effect on the interaction of parasite and host that figures for percentage parasitism, etc., have little significance outside the peculiar circumstances occurring at the point in time and space where the sample was taken, and again description in general terms gives a better indication of the effectiveness of a parasite and its possible value on introduction to a new area where its reactions to the environment are uncertain. What has been said above about sampling to obtain an estimate of parasite activity *before* introduction into a new area is equally true when considering the effectiveness of the control exerted by a parasite species *after* introduction. Here, however, from a purely practical standpoint, if the degree of control exerted warrants the expense of introduction the effectiveness of the species is evident in an economic reduction of the pest in question.

SOME FURTHER ISOLATED GENERATIONS OF TSETSE FLIES.

By C. H. N. Jackson.
Department of Tsetse Research, Tanganyika.

Methods and Objects.

In August, 1946, four more liberations of tsetse flies were made by exposing the pupae for three days in the habitats of related species. From dawn on 17th to dawn on 20th, about 30,000 pupae of *Glossina morsitans*, Westwood, were exposed at the same point as in the previous year (Jackson, 1946), in the habitat of *G. swynnertoni*, Austen, and *G. pallidipes*, Austen ; and simultaneously about 27,500 pupae of *G. swynnertoni* were exposed in the habitat of *G. morsitans* in eastern Kahama District. A week later, about 20,000 pupae of each species were exposed together in the habitat of *G. pallidipes* at Shinyanga in an isolated block of bush about five square miles in area, from which *G. swynnertoni* had almost or quite disappeared. These later exposures were from dawn on 24th to dawn on 27th August.

The *morsitans* emerging from the releases on 17th–20th were recaptured on the same " squarely spiral " route (30,000 yards) as in the previous year, and the *swynnertoni* emerging at the same time were simultaneously recaptured on an exactly similar " spiral ". The searches for first generation flies and their offspring were continued to the 91st day, except in the case of the *swynnertoni* released in *morsitans* country, where no second generation was seen and catches ceased on the 77th day. The flies of both species emerging on 24th–27th August were recaptured on a regular but not " spiral " route close to the release site and covering the isolated block more or less evenly ; this route was 10,000 yards long, and was followed till the 91st day. It was estimated from counts of pupae and empty cases that about 4,000 flies of each species emerged during the releases of 17th–20th, and about 3,000 of each during those of 24th–27th. The flies were all collected by parties of three individuals daily, provided with attractant black screens ; they were dealt with exactly as in the previous year (Jackson, 1946), and control flies of the indigenous species were similarly treated.

The objects were to shed further light on the reasons why each species favours its particular habitat (Jackson, 1945a) ; to investigate the reason for delayed maximal catch until about the third week after release despite early deaths ; to check the previous data on the relation of age to wing-fray ; to find whether, as had been suspected before, smaller flies were really less viable ; and to throw further light on the reproductive cycle.

Comparison of the Species.

Table I shows mean daily weights in milligrams of water, fat and residue of first generation males recaptured, day 0 being respectively 18th and 25th August in the two pairs of experiments. In Table II the means of all the males recaptured are compared with the means of control indigenous males caught at the same time, and with the means of freshly emerged males under 24 hours old.

Comparison with the 1945 release of *morsitans* (Jackson, 1946) shows that, in the same place in 1946, recaptures were little more than a third of the 1945 total, when the release was done rather earlier in the season (21st July), but the numbers of recaptures of teneral (unfed) flies were about the same—44 in 1945 and 50 in 1946. The mean weight of fat in the males was 2·9 mg. in weeks 3 and 4 (when it had reached

TABLE I.

Daily analysis of first generation males released.
m = morsitans, s = swynnertoni, p = pallidipes.

Released Habitat of	m s&p	m p	s m	s p	m s&p	m p	s m	s p	m s&p	m p	s m	s p	m s&p	m p	s m	s p
Day	Water				Fat				Residue				Numbers			
0	11·6	13·3	17·5	11·2	1·6	1·2	0·9	0·9	5·0	4·4	4·5	4·2	1	2	6	3
1	Sunday															
2	13·4	12·2	16·0	11·0	1·1	1·3	0·8	0·8	4·9	4·9	4·3	4·4	9	13	38	8
3	12·7	11·4	12·0	11·6	1·0	0·7	0·7	0·8	4·9	4·8	4·5	4·6	9	5	36	8
4	12·8	9·4	10·9	9·3	0·8	1·3	0·6	0·7	4·8	4·5	4·5	4·2	5	3	26	7
5	13·4	17·6	12·8	11·6	0·9	2·3	0·5	0·9	4·9	7·1	4·2	5·5	2	1	25	1
6	16·5	13·1	10·7	—	1·9	0·8	0·6	—	6·6	5·3	4·5	—	1	1	18	0
7	14·8	11·9	13·5	11·3	1·6	0·8	1·1	1·0	6·0	5·3	5·7	5·2	2	1	9	2
8	Sunday															
9	15·7	16·6	14·4	—	2·1	2·7	1·5	—	6·5	6·8	5·8	—	4	3	4	0
10	15·8	12·0	13·2	17·5	1·9	1·3	1·5	2·9	6·6	5·5	5·7	7·6	5	2	6	4
11	18·6	16·9	15·6	15·0	2·3	1·8	1·7	2·5	8·3	7·0	6·2	6·4	6	2	7	7
12	16·1	15·7	16·4	18·1	2·1	2·8	2·2	3·8	7·3	6·3	6·6	7·7	6	1	10	4
13	16·4	16·0	16·7	15·4	3·3	2·3	2·5	2·6	7·4	6·8	6·8	6·7	2	2	9	23
14	17·5	15·2	15·3	16·2	2·1	2·1	2·2	2·7	7·5	7·0	6·7	7·2	3	3	7	18
15	Sunday															
16	16·9	15·8	15·6	15·5	2·1	2·6	2·1	2·5	7·6	7·9	6·9	6·8	4	2	14	12
17	18·5	17·2	19·2	15·2	2·5	2·1	2·9	2·5	7·9	7·5	7·8	7·0	5	7	10	18
18	15·8	17·2	16·4	15·0	3·1	2·6	2·0	2·2	7·1	7·4	6·9	6·9	6	5	8	15
19	16·2	14·4	18·0	15·7	2·3	2·2	3·0	2·6	7·4	6·8	7·1	7·0	5	4	3	6
20	14·9	17·3	16·8	17·8	1·7	3·2	3·2	2·3	6·8	6·7	7·1	6·0	3	2	1	6
21	16·3	15·4	15·8	15·3	2·9	2·5	1·5	3·1	6·8	6·6	6·5	6·8	1	5	3	4
22	Sunday															
23	15·2	15·3	17·8	16·7	1·9	2·2	2·1	2·5	7·1	6·8	7·2	7·3	2	3	3	1
24	19·7	18·5	16·5	—	3·3	3·4	2·5	—	8·4	7·5	7·0	—	3	3	2	0
25	16·4	16·4	15·1	17·7	2·9	2·0	1·4	2·7	7·6	6·8	6·5	6·2	3	2	1	6
26	16·3	15·9	16·0	18·0	3·0	3·4	2·2	3·5	7·2	7·3	7·0	7·5	3	2	2	3
27	16·5	17·4	16·6	—	3·0	2·9	1·9	—	7·2	7·9	7·0	—	4	1	2	0
28	—	17·4	12·6	—	—	2·8	1·0	—	—	7·7	6·3	—	0	3	1	0
29	Sunday															
30	—	15·2	17·4	—	—	2·0	3·5	—	—	7·0	7·1	—	0	2	3	0
31	17·3	—	15·4	14·3	2·3	—	2·0	1·6	7·6	—	7·3	6·9	3	0	1	3
32	18·6	—	24·9	15·6	2·6	—	3·2	2·4	8·3	—	12·0	6·8	1	0	1	2
33	15·6	—	—	18·4	2·3	—	—	0·7	7·3	—	—	6·8	2	0	0	1
34	16·2	15·6	—	17·0	2·4	1·6	—	2·8	7·7	7·8	—	7·2	5	1	0	1
35	17·9	18·6	18·9	—	3·1	2·2	3·3	—	7·5	7·4	7·8	—	1	2	1	0
36	Sunday															
37	14·6	17·1	17·2	18·1	1·1	1·9	3·2	0·7	7·0	7·2	7·0	6·1	2	1	1	1
38	15·7	—	—	23·2	2·1	—	—	2·2	7·2	—	—	7·6	1	0	0	2
39	19·2	—	—	—	3·6	—	—	—	8·8	—	—	—	2	0	0	0
40	—	—	—	17·4	—	—	—	2·5	—	—	—	7·1	0	0	0	1
44	12·0	—	19·2	15·9	0·6	—	3·9	2·5	6·2	—	10·0	6·6	1	0	1	2
45	16·8	—	15·4	20·1	1·3	—	2·3	2·9	7·4	—	6·0	7·2	1	0	1	1
53	—	17·4	—	—	—	3·3	—	—	—	7·8	—	—	0	1	0	0
61	—	22·0	—	—	—	3·5	—	—	—	8·5	—	—	0	1	0	0
63	—	—	—	16·4	—	—	—	2·3	—	—	—	6·8	0	0	0	1
66	—	—	—	15·2	—	—	—	2·8	—	—	—	7·2	0	0	0	1
Total ...	15·7	15·1	13·3	15·0	2·1	2·1	1·3	2·3	6·8	6·5	5·4	6·5	112	84	254	169

Day 0 is not included in the totals.

a maximum) in 1945, compared with 2·7 mg. in these weeks in 1946, and the weights of water and residue were lower, and the proportion of non-teneral females higher, in 1946. Whether the poorer condition and poorer survival of the *morsitans* in 1946 can be entirely attributed to the later date of the release is doubtful ; it is suggestive that teneral flies emerging in the laboratory in August, 1946 (Table II) weighed only 19·9 mg. compared with 22·8 mg. for similar flies in late July—early August, 1944, and their fat content averaged only 1·39 mg. compared with 1·62 mg. just over two years earlier (Jackson, 1945, Table V). In the interval there had also been a marked fall (from May, 1945) of numbers of *G. swynnertoni* caught on routine rounds in Shinyanga, but unfortunately comparable figures for *morsitans* populations are lacking.

TABLE II.

Means of recaptured males and controls.

Species	Habitat of	Water	Fat	Residue	Numbers
Teneral laboratory controls under 24 hours					
morsitans	—	13·74	1·39	4·79	121
swynnertoni	—	12·61	1·02	4·52	107
pallidipes	—	17·60	2·32	6·30	20
Teneral flies at large					
morsitans	*morsitans*	11·55	·79	4·76	41
	swynnertoni ...	12·68	·99	4·87	26
	pallidipes	11·62	1·12	4·77	24
swynnertoni	*morsitans*	10·96	·64	4·35	146
	swynnertoni ...	10·50	·63	4·38	38
	pallidipes	10·79	·87	4·46	28
pallidipes	*pallidipes*	14·51	1·05	6·20	28
Non-teneral flies at large					
morsitans	*morsitans*	17·16	2·30	7·48	114
	swynnertoni ...	16·63	2·39	7·40	87
	pallidipes	16·48	2·43	7·13	60
swynnertoni	*morsitans*	16·35	2·16	6·84	109
	swynnertoni ...	15·77	2·31	7·11	89
	pallidipes	15·84	2·56	6·88	141
pallidipes	*pallidipes*	20·29	1·16	9·82	75
morsitans 1945 for comparison (habitat of *swynnertoni*)					
	Teneral	12·98	1·10	5·46	28
	Non-teneral ...	18·00	2·44	7·98	233

In 1944 it had been observed that first generation *morsitans* released in the habitat of *swynnertoni* were about the same weight as the indigenous species after allowing for the difference in size, but that they were considerably lighter in all respects than *morsitans* in its own home (Jackson, 1945a). In the 1946 experiments, however, this was not so, for released non-teneral *morsitans* males averaged 2·4 mg. fat compared with 2·3 mg. for native *morsitans* caught at the same time, and the weights of water and residue were only a little higher among the native flies (Table II). It may be that the habitat of *morsitans* selected in eastern Kahama was not really very favourable, though it did include some *Brachystegia* woodland usually characteristic of the *morsitans* country.

It is evident from Table II that teneral male *swynnertoni* in the laboratory were almost exactly 91 per cent. of the weight of *morsitans*, which in turn were almost exactly 76 per cent. of the weight of *pallidipes*. In Table III, therefore, the weights of *swynnertoni* are multiplied by 1·1 and the *pallidipes* by ·76, in order to make them comparable with the weights of *morsitans*. When this is done, all the non-teneral *swynnertoni* weights—water, fat and residue—are higher than those of *morsitans*, with the single exception of the fat in *swynnertoni* released in the *morsitans* habitat ; but this is not true of the teneral males, which suggests that *swynnertoni* is able to build up its reserves better from the start. Except in residual dry weight, non-teneral *pallidipes* are considerably poorer than the other two species ; it is well known that *pallidipes* is normally very hungry when it comes to the catchers.

The non-teneral male *swynnertoni* are actually heavier in the habitat of *morsitans* or of *pallidipes* than in their own home, where, however, they have the highest residual dry weight. This is extremely puzzling, and quite opposed to the experience with *morsitans* in 1943–44 (Jackson, 1945a).

TABLE III.

As for Table II, but *swynnertoni* and *pallidipes* corrected for difference in size from *morsitans*.

Species	Habitat of	Water	Fat	Residue	Total
Teneral laboratory controls under 24 hours					
morsitans	—	13·74	1·39	4·79	19·92
swynnertoni	—	13·87	1·12	4·97	19·96
pallidipes	—	13·38	1·76	4·79	19·93
Teneral flies at large					
morsitans	*morsitans*	11·55	·79	4·76	17·10
	swynnertoni ...	12·68	·99	4·87	18·55
	pallidipes	11·62	1·12	4·77	17·51
swynnertoni	*morsitans*	12·05	·71	4·78	17·54
	swynnertoni ...	11·55	·69	4·81	17·05
	pallidipes	11·87	·96	4·90	17·73
pallidipes	*pallidipes*	11·03	·80	4·71	16·54
Non-teneral flies at large					
morsitans	*morsitans*	17·16	2·30	7·48	26·94
	swynnertoni ...	16·63	2·39	7·40	26·42
	pallidipes	16·48	2·43	7·13	26·04
swynnertoni	*morsitans*	17·98	2·38	7·53	27·89
	swynnertoni ...	17·35	2·54	7·82	27·71
	pallidipes	17·42	2·81	7·57	27·80
pallidipes	*pallidipes*	15·42	·88	7·47	23·77

These results are obviously inexplicable at present. Meanwhile comment may be made on one quite new point which emerges from a study of Table III. It appears that among non-teneral flies at large in their own habitat, the smaller the species the higher will be its mean weight in relation to the weight at emergence. In Table IV, the wet weights of non-teneral males in their own habitats have been expressed as increments on the wet weights of flies under 24 hours old. Only one non-teneral *swynnertoni* male was below the average weight at emergence, and that was ·99 of the average emergence weight; but four *morsitans* were respectively ·86, ·87, ·96 and ·98 of the emergence weight, and no less than 19 *pallidipes*, a quarter of the total, were below it, the lightest being only ·64 of the mean weight at emergence. It might, therefore, appear that if *morsitans* in these experiments sometimes seems more hungry than *swynnertoni*, this may be because some of the very hungry *morsitans* correspond to *swynnertoni* which, because of their smaller size, are unable to survive at such relatively low weights, and being cut off by death are not represented in the samples caught.

TABLE IV.

Wet weights of non-teneral male *morsitans*, *swynnertoni* and *pallidipes* from their own habitats, expressed as increments on wet weights of laboratory controls under 24 hours old.

Increment	*swynnertoni*	*morsitans*	*pallidipes*
·6–	—	—	1
·7–	—	—	2
·8–	—	2	3
·9–	1	2	13
1·0–	8	10	13
1·1–	9	16	9
1·2–	19	16	15
1·3–	11	22	13
1·4–	12	24	2
1·5–	9	6	1
1·6–	12	4	—
1·7–	5	3	—
1·8–	3	8	1
1·9–	—	1	1
2·3–	—	—	1
2·5–	—	—	1
—	89	114	76

Non-teneral males weighing as much as or more than the mean weight at emergence include 47 per cent. weighing 1·4 or more times the mean emergence weight in *swynnertoni*, 42 per cent. in *morsitans* and only 12 per cent. *pallidipes*. The last figure is very low because, as already remarked, *pallidipes* that are not hungry are not usually attracted to the catchers, whereas many *morsitans* and *swynnertoni* that are not hungry at all attend the catching party on the look-out for females.

Early Inactivity of Males.

Table V shows the time of capture of the released flies, divided into teneral and non-teneral flies of each sex. It is quite clear that, as before (Jackson, 1946), there is no marked tendency for the non-teneral captures to fall away until the fourth week, although when *swynnertoni* were released in the habitat of *morsitans*, more than half the total catch was tenerals caught in week 1. Whether in this particular experiment death was exceptionally heavy in the early stages it is impossible to say; Table VI shows that dispersal was much more rapid than on the other "spiral" of the same size, and many flies may have eluded capture by passing out of reach.

In the 1946 experiments, an additional length of catching path, 150 yards long, was run from the inner end of each "spiral" right up to the release site; the flies

TABLE V.

Weekly catches of first generation *morsitans* (m) and *swynnertoni* (s) in habitat of alternate species or of *pallidipes* (p). T signifies teneral and NT non-teneral flies.

Released ...	m		m		s		s		m		m		s		s	
Habitat of ...	s & p		p		m		p		s & p		p		m		p	
	Males								Females							
Week	T	NT	T	NT	T	NT	T	NT	T	NT	T	NT	T	NT	T	NT
1	25	3	22	2	143	9	25	1	25	3	6	3	125	5	10	6
2	0	26	1	12	0	43	0	56	2	13	0	3	0	16	0	1
3	0	24	0	25	0	39	0	61	0	3	0	0	0	9	0	2
4	0	15	0	14	0	11	0	10	0	5	0	0	0	7	0	0
5	0	12	0	5	0	6	0	7	0	2	0	1	0	1	0	4
6	0	5	0	1	0	1	0	4	0	4	0	0	0	1	0	1
7	0	2	0	0	0	2	0	3	0	1	0	0	0	1	0	0
Later	0	0	0	2	0	0	0	2	0	3	0	0	0	1	0	1

caught on these extra paths were very few, and have been omitted from Table I-VIII, but included, together with some females from extra catches, in Table IX. In the releases on 24th–27th August the catching path was arranged to pass close by the release site. It is therefore clear that, since some flies must be dying from the start, low catches in weeks 1 and 2, or failure to fall from week 1 to week 3, are not accounted for by flies failing to leave the release site, and must be explained by relative inactivity of males when they are fairly young. This is an important point, bearing on the interpretation of estimated population and length of life from marked fly data.

TABLE VI.

Dispersal of males in yards by weeks, compared with 1945 results. The values tend asymptotically towards 1450, when all sectors of the spiral would show equal density of recaptures.

Week	*morsitans* in *swynnertoni* country		*swynnertoni* in *morsitans* country
	1945	1946	1946
1	365	573	1,099
2	484	724	823
3	772	969	1,100
4	941	1,875	1,309
5	995	1,288	1,375
6	1,259	930	900
7	838	1,275	1,575
8	1,000	—	—
9	1,312	—	—

The females, once again, behave in the opposite way, for they are relatively inactive in the latter part of their lives; though few are seen later, they do not die quickly, and always form a substantial proportion of the longest-term recaptures.

The Relation of Wing-fray to Age.

Table VII shows the result of placing the male recaptures in six arbitrary categories of wing-fray, and comparing their actual ages with those deduced from the relevant tables previously given (Jackson, 1946). The diagnosis was again performed by my assistant Mrs. Zieskowicz, and she measured the wing veins discussed below.

TABLE VII.

Age of males deduced from numbers assigned to each arbitrary category of wing-fray, compared with 1945 result and with actual age in days.

Species released ...		*morsitans*			*swynnertoni*	
In habitat of		*swynnertoni*		*pallidipes*	*morsitans*	*pallidipes*
Year		1945	1946	1946	1946	1946
Age-group	Mean					
1–5	3	8	6	12	6	5
6–10	8	10	8	11	6	7
11–15	13	9	19	13	7	13
16–20	18	18	19	15	8	15
21–25	23	22	30	19	8	18
26–30	28	27	29	28	12	24
31–35	33	32	44	50	17	32
36–40	38	38	39	35	14	35
41–45	43	47	57	—	35	29
46–50	48	49	—	—	—	—
51–55	53	52	—	57	—	—
56–60	58	57	—	—	—	—
61–65	63	57	—	—	—	57
66–70	68	—	—	—	—	57

(The minimum possible value is 5 and the maximum 57).

Three out of the four experiments done in 1946 show fair agreement with the 1945 results ; but the *swynnertoni* released in the habitat of *morsitans* are quite aberrant in the very slow fraying of the wings. This is the more surprising because (Table VI) they dispersed remarkably quickly, and it might have been expected that their wings would show heavy wear.

Later in the same year, an attempt was made to find whether *morsitans* wears its wings at a different rate during the rainy season, by collecting pupae at the end of the dry season (after which they are difficult to find) and exposing them for three days in the habitat of *swynnertoni* during the early rains. The rains broke early and not enough pupae were collected, but such results as are available do not indicate a result very different from those observed in the dry season.

TABLE VIII.

Pooled results of the 1946 releases, but omitting the release of *swynnertoni* in *morsitans* habitat ; showing deduced ages of males from wing-fray compared with actual age in days, and effect of treating females by the same formula as the males.

Age-group	Mean Age	Deduced age, males	Females	Numbers	
				Male	Female
1–5 ...	3	7	9	70	50
6–10 ...	8	9	13	25	21
11–15 ...	13	15	20	77	9
16–20 ...	18	16	18	100	20
21–25 ...	23	22	19	33	15
26–30 ...	28	27	19	18	10
31–35 ...	33	39	21	22	10
36–40 ...	38	37	27	9	10
41–45 ...	43	43	18	4	4
46–50 ...	48	—	23	0	1
Total ...	16	17	—	358	—

Table VIII shows the result of applying the same formula to the females released in these experiments, and supports the previous conclusion (Jackson, 1946) that, in the later weeks at least, the females wear their wings less rapidly than do the males.

The Relation of Vialibility to Size.

As a measure of size, the middle part of the 4th longitudinal vein (Jackson, 1945a) is used. In 1944, it was found that teneral male *morsitans* released from pupae had a significantly smaller mean vein measurement than non-teneral males from the same release. As it was also observed that smaller teneral males exposed in the laboratory under constant conditions for 24 hours lost a greater percentage of their weight than did larger ones, and the smaller species, *swynnertoni*, lost a greater percentage than the larger species, *morsitans*, it was supposed that smaller flies would be relatively non-viable in the field. In 1945, admittedly, no significant difference was found between the mean vein lengths of teneral and non-teneral flies recovered, but the numbers of tenerals especially were small (Jackson, 1946).

From the 1946 releases, there are available, from both species and both sexes, 994 recaptures of which 439 are teneral (mostly *swynnertoni* in the habitat of *morsitans*) and 555 are not. The two sexes of each species separately are shown in Table IX. In three cases out of the four, the non-teneral flies have the longer mean vein, and a χ^2 test shows that the four groups are not statistically heterogeneous in this respect. I am grateful to Dr. F. Yates of Rothamsted for carrying out this test, and for guidance in the treatment of the data generally.

TABLE IX.

Increase in mean length, in hundredths of a millimeter, of the middle part of the fourth longitudinal vein, from teneral (T) to non-teneral (NT) males and females of *G. morsitans* (m) and *G. swynnertoni* (s), allowed to emerge from pupae and afterwards recaptured in the field.

Species	Sex	T/NT	Mean	S.E.	Flies	$\frac{1}{(S.E.)^2}$	Weighted difference
m	♂	T	149·8240	±·6888	63		
		NT	149·5171	±·3635	161		
	Increase		—·3069	±·7788		1·648	—·5059
	♀	T	169·0766	±·9622	48		
		NT	169·9356	±·8457	69		
	Increase		+·8590	±1·2890		·602	+·5171
s	♂	T	142·2878	±·3369	199		
		NT	143·0769	±·3109	249		
	Increase		+·7891	±·4593		4·740	+3·7403
	♀	T	159·0734	±·4847	129		
		NT	160·5208	±·6682	76		
	Increase		+1·4474	±·8254		1·452	+2·1015
				Total ...	994	8·442	+5·8530
					Reciprocal	·1185	
				Divide by 8·442 ...	...	...	+·6934
				$\sqrt{\cdot 1185}$	...	...	±·3442
				t	...	...	+2·015

The teneral flies have a weighted mean vein-length ·006934 mm. less than the non-tenerals, and the standard error of this difference is ±·003442. The difference thus corresponds to a probability of about 1 in 20, and the probability of the non-tenerals being the greater (as forecast) is therefore about 1 in 40, which is fairly conclusive.

Although the actual difference between the means of tenerals and non-tenerals is only ·0069 mm., about the diameter of a human erythrocyte, in order to obtain such a shift in the mean it would be necessary to cut off about 10 per cent. of all the teneral flies from the short-vein end of the curve of distribution. It therefore appears that during August about one in ten of those released in an alien habitat is too small to survive long enough to obtain its first meal and appear as a non-teneral fly. This is a very striking result and suggests that small differences in size of larvae and pupae from year to year have a marked effect on the size of tsetse population.

There is no suggestion from the data that smaller non-tenerals are less viable, from which it may be supposed that, once a fly has taken its first meal, it is in little danger of starvation.

The difference in mean vein length of tenerals and non-tenerals is of the order of difference between successive monthly means of non-teneral males, which vary in a very regular way with the season, the smallest flies appearing about November or December and the largest in June.

The External Morphology of first and second Instar Larvae.

In studying the reproductive cycle, it was necessary to distinguish the larval instars, and as the published accounts do not agree, the following notes, based on the examination of large numbers of young larvae dissected out alive and afterwards preserved in polyvinyl alcohol-lactophenol, are given.

The first-instar larva is usually from 1·4 to 1·8 mm. long. The integument is covered with minute spines from 1 to 3 microns in length. The respiratory lobes are undeveloped (Roubaud, 1909) and the tracheal trunks open by two funnels flush with the surface. The mouth is not functional at this stage ; it is blocked by a strong egg-tooth about ·05 mm. long, which, together with the spines, was apparently overlooked by Roubaud.

The second-instar larva measures from about 1·8 to 4·2 mm. Like the first instar, it is covered with small spines, not mentioned by Roubaud, but described and figured by Newstead, Evans and Potts (1924). The respiratory lobes are somewhat projecting though not yet well separated as in the third instar, and between them are rows of tapered spines up to 12 microns in length posteriorly, but decreasing in size from behind forwards. The antenno-maxillary complex of the third instar is undeveloped.

The third-instar larva, with its characteristic " shagreened " integument and no spines, has been described and figured by various authors. According to my colleague Mr. W. H. Potts, the respiratory lobes of the third instar blacken about 1½ days before larviposition. The larva is then commonly about 7 mm. long, and the antenno-maxillary complex and anus are also black.

It is evident that Newstead, Evans and Potts did not see a first-instar larva and the specimen figured by them is certainly early second-instar. No trace of the long spatulate hairs figured by them has been found, and apparently they did not find them in later second-instar larvae.

The Reproductive Cycle.

According to Helen Mellanby (1937), ovulation in *G. palpalis*, R.-D., occurs within 24 hours of the expulsion of the previous larva, and the egg remains for three days in the uterus before hatching at 24°C. The larval period is 5 or 6 days, and 10 days is the normal period between successive larvae at 24°. According to Jack (1939), however, the interlarval period for *G. morsitans* at 24° is 11 days. There is no precise information about the time required for production of the first larva, which appears in about 19 days if flies are offered food daily, but if food is offered at 3-day intervals the larvae are larger and take about 23 days.

Fertilisation occurs after ovulation, as Stuhlmann (1907) surmised. Sections of eggs from the ovaries show no sign of development even in flies which have just deposited a larva or which contain a black-lobed larva.

There is some difficulty in interpreting results from the release of flies in the habitat of related species, because their own males are not potent for a few days, and the indigenous males may fail to inseminate the alien females, even though, as is the case with *morsitans* and *swynnertoni*, they copulate freely in the field (Jackson, 1945b). In the 1946 releases, females of both *morsitans* and *swynnertoni* released in each other's habitats or in that of *pallidipes* were often not inseminated or only slightly inseminated up to well into the second week, after which nearly all were fully inseminated, from which we may suppose that if insemination fails at the first copulation they may copulate again until full insemination is effected. The failure to inseminate evidently results very often in the loss of the sterile first egg from the right ovary, and early ovulation from the left, because no fewer than eleven ovulations from the left ovary were found on days 19 and 20, too soon to have followed larvae from the right.

In all the 1946 releases combined, there was a batch of nine ovulations from the right ovary between days 11 and 20, and from these there were a first-instar larva on day 17, a second instar on day 18, an early third instar (moulting) on day 19, and a large white-lobed larva on day 23. Mean screen temperature during this time was about 24°C., so that these results are in keeping with the idea (Jackson, 1946) that the flies were living at about the temperature of the standard climate. There were, however, two anomalous first-instar larvae on days 22 and 25.

The batch of ovulations from the left ovary starting on day 19 continued to day 27, during which time 21 ovulations were found, and from these, first-instar larvae were seen on days 23, 24 and 26; there was also a second-instar larva on day 23, and black-lobed third-instar larvae were seen on days 26 and 30. All these larvae from the left ovary seem too early to have followed the deposition of a larva from the right, and they probably followed the loss of a sterile egg.

The next batch of ovulations from the right ovary occurs about day 30, and from the left about day 40. Only five more larvae were seen; these were first instars from the right on days 35 and 41, and second instars from the right on days 38 and 51, and from the left on day 67.

Apart from the two anomalous first instars on days 22 and 25, the larvae from the right ovary are fairly well up to time for a temperature of 24°C.; and as the first ovulations from the left ovary began abruptly on day 19, the larvae resulting developed quite as quickly as would be expected. It was therefore concluded that the females are living at about the temperature of the standard climate, rather than in specially selected, cooler ecoclimates.

The first teneral fly of the second generation appeared on day 53, and three more on days 55 and 56, after which a further eighteen were caught to day 91. Teneral flies average about 3 days old when caught, so that if the larvae producing the first ones had pupated about day 23, the pupal period would have been about 27 to 30 days. During this time the mean screen temperature was about 24½°C., and such a pupal period would be in accordance with Jack's (1939) results, if, on the other hand, the first larvae were deposited a little later than day 23, as is possible, then the flies would be living slightly below the temperature of the standard climate and the pupae slightly above it.

Summary.

Glossina morsitans and *G. swynnertoni* were allowed to emerge for three days from pupae placed in each other's habitats and in that of *G. pallidipes*.

The *morsitans* in the habitat of *swynnertoni* were in poorer condition, and survived less well, than in the previous year. This may have been because the release was done a little later in the season, or because the freshly emerged flies were not up to the nutritional standards of a year or two before.

They were, however, nearly as heavy, in later life, as wild *morsitans* in their own home, and this is contrary to previous experience.

In relation to initial weight, *swynnertoni*, whether in their own home or as aliens in the habitats of other species, were heavier than *morsitans*, and in fat and water the alien *swynnertoni* were heavier than the native ones. However, the difference between the two species may possibly be caused by the fact that *swynnertoni*, after taking its first meal, cannot apparently fall much below its initial weight at emergence, as *morsitans* and particularly *pallidipes* are able to do, so that very hungry *morsitans* may be represented by *swynnertoni* which have been cut off by death, and are not available to the catchers.

The males of *morsitans* and *swynnertoni* are relatively inactive in early life, which has an important bearing on estimates of population and mean life made from marked flies. The females on the contrary are relatively inactive in later life.

Generally the relation between wing-fray and age found in the previous year is confirmed, except one aberrant result when *swynnertoni* was released in the habitat of *morsitans*. Scanty data suggest that the rate of wing-fray during the rainy season is not very different. As before, the females, especially in later life, fray their wings more slowly than the males.

Teneral (unfed) flies below the average size are comparatively non-viable, and about 10 per cent. are too small to survive to obtain their first meal. There is no suggestion that the same applies to non-tenerals.

The external morphology of early larvae is described ; the first instar is provided with an egg-tooth in the mouth, which makes it incapable of feeding. Both the first and the second instars are spiny.

Although copulation between *morsitans* and *swynnertoni* is random, insemination from interspecific matings often fails, and the sterile egg is aborted. In such cases the left ovary ovulates before the normal time.

The flies and pupae live at about the temperature of the standard climate as recorded in a Stevenson screen ; possibly the flies are a little cooler and the pupae a little hotter.

References.

JACK, R. W. (1939). Mem. Dep. Agric. S. Rhod., no. 1, 203 pp.

JACKSON, C. H. N. (1945a). J. Anim. Ecol., **14**, pp. 46–51.

——. (1945b). Proc. R. ent. Soc. Lond., (A) **20**, p. 106.

——. (1946). Bull. ent. Res., **37**, pp. 291–299.

MELLANBY, H. (1937). Parasitology, **29**, pp. 131–141.

NEWSTEAD, R., EVANS, A. M. & POTTS, W. H. (1924). Guide to the Study of Tsetse Flies. London.

ROUBAUD, E. (1909). La *Glossina palpalis*. *In* Martin, G., Leboeuf & Roubaud, E. Rapport de la Mission d'Etudes de la Maladie du Sommeil au Congo Français, 1906–1908, pp. 383–643. Paris.

STUHLMANN, F. (1907). Arb.GesundhAmt., **26**, pp. 301–382.

NOTES ON THE BIONOMICS OF *ANOPHELES GAMBIAE* AND *A. FUNESTUS* IN EAST AFRICA.

By K. S. Hocking and D. G. MacInnes.

In the course of certain applications of Anopheline control measures by military malaria units in East Africa, including the use of residual DDT and airspraying with DDT, many of the gaps in our knowledge of Anopheline behaviour became only too apparent. The limitations placed upon the intelligent application of these measures by our lack of knowledge justified the investigations here recorded. At the same time the nature of the observations explains their apparent disconnectedness as well as their incompleteness.

Time of Entry into Huts and Tents.

The mosquitos in several small mud huts and bush tents near Taveta in Kenya were collected at three-hour intervals during the night on 51 occasions between November, 1944, and June, 1945, with the following results. (See also fig. 1.)

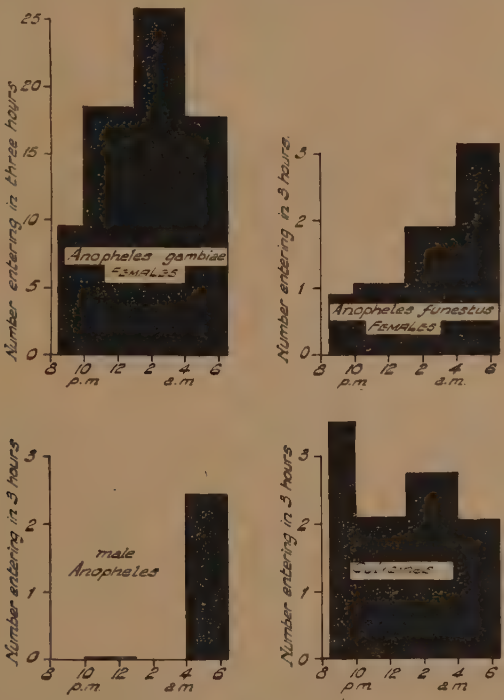


Fig. 1.—Times of entry of mosquitos into huts and tents.

Anopheles gambiae females.

A total of about 5,000 female *A. gambiae* entered as follows :—

	8.30 to 10.0 p.m.	10 p.m. to 1 a.m.	1 a.m. to 4 a.m.	4 a.m. to 6.30 a.m.
Total number entering ...	650	1,482	1,679	1,094
Entry per night per 3 hours (geometric mean)	9·67	18·50	25·74	17·74
"t" test for significance ...		P= ·02 (significant)	P= ·05 (significant)	P= ·1 (not significant)

Haddow (1942) at Kisumu, Kenya, found a large resting population of *A. gambiae* females at 7 p.m., a period of low entry from 8 to 10 p.m., of high entry from 11 p.m. to 5 a.m. and a sharp entry peak at 6 a.m.

Our results agree with Haddow's findings except that there is no evidence of an entry peak at 6 a.m. In our experiments the resting population had been destroyed by the previous morning's spraying.

Anopheles funestus females.

During the period of these experiments, the numbers of *A. funestus* were much lower than those of *A. gambiae*, but the results agree very well with those of Haddow, who, at Kisumu in 1942, found a large resting population at 7 p.m., a period of low entry from 8 p.m. to 2 a.m., of higher entry from 3 to 5 a.m. and an entry peak at 6 a.m. De Meillon in South Africa, on the other hand, found that the highest entry was at 11 p.m.

	8.30 to 10.0 p.m.	10.0 p.m. to 1 a.m.	1 a.m. to 4 a.m.	4 a.m. to 6.30 a.m.
Total number entering ...	34	62	92	250
Entry per night per 3 hours (geometric mean)	0·91	1·04	1·92	3·15
"t" test of significance ...		(Not significant)	P= ·05 (significant)	P= ·05 (significant)

Male *Anopheles*.

Of the 324 male *Anopheles* entering our tents and huts during the night catches, three entered between 10 p.m. and 1 a.m., one between 1 and 4 a.m. and 320 after 4 a.m.

De Meillon (1933) in South Africa found that males started to enter at 1 a.m. with a peak of entry at 5 a.m. Haddow in Kenya recorded that males entered at 6 a.m.

Culicines.

Culicine mosquitos (*Culex* and *Taeniorhynchus* spp.) entered in greatest numbers early in the night, but continued to enter in fairly constant numbers throughout the night.

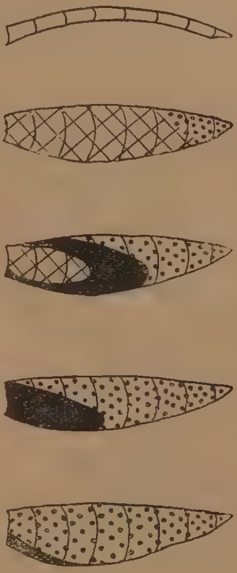
	8.30 p.m. to 10 p.m.	10 p.m. to 1 a.m.	1 a.m. to 4 a.m.	4 a.m. to 6.30 a.m.
Total number entering ...	185	210	236	218
Entry per night per 3 hours (geometric mean)	3·49	2·08	2·74	2·05

Ovary Development.

In order to study ovary development, it was first necessary to determine the correlation between follicle development and external morphology. For this purpose, follicle development was based on the five stages recognised by Covell (1939), which may be expressed briefly as follows :—

Stage I. Follicle without yolk granules.
,, II. Yolk granules occupying less than half of follicle.
,, III. Yolk granules occupying more than half of follicle.
,, IV. Follicle elongate ; floats not developed.
,, V. Egg fully developed, with floats.

The following external characters were found to correspond with these stages, and were therefore adopted for subsequent observations :—



Stage I.—No visible ovaries ; no blood.

,, II.—Abdomen more or less distended ; ovaries occupying not more than 3 abdominal segments dorsally and 2 segments ventrally.

,, III.—Ovaries occupying not more than 6 segments dorsally and 4 ventrally.

,, IV.—No visible red blood ; ovaries occupying all but 0–1 segments dorsally, and occupying 3–4 ventrally.

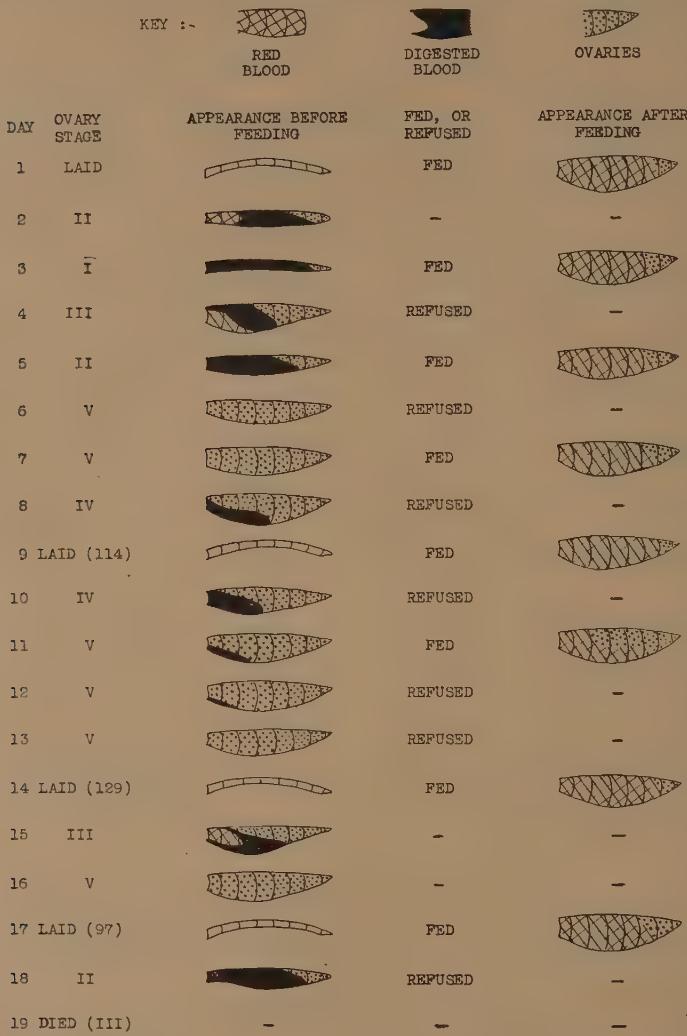
,, V.—Stomach not visible, or visible only as a narrow black line on the ventral surface.

Fig. 2.

Senior White, Ghosh and Venkat Rao (1945) have shown that in various Oriental species of *Anopheles* there is a close correspondence between the ovarian and external stages, with the exception of stage I.

Procedure.

Stage V females of both *A. gambiae* and *A. funestus* were collected and isolated in cages, each with a bowl of water containing a few twigs and grasses. After laying, daily observations were made, and the appearance of the abdomen recorded diagrammatically; in most cases the insect was then offered food, and the abdominal appearance after feeding similarly recorded. A typical series of such diagrams of each of the diagrams of each of the vector species is reproduced in figures 3 and 4 to illustrate



DAY	OVARY STAGE	APPEARANCE BEFORE FEEDING	FED, OR REFUSED	APPEARANCE AFTER FEEDING
1	LAID		FED	
2	II		–	–
3	I		FED	
4	III		REFUSED	–
5	II		FED	
6	V		REFUSED	–
7	V		FED	
8	IV		REFUSED	–
9	LAID (114)		FED	
10	IV		REFUSED	–
11	V		FED	
12	V		REFUSED	–
13	V		REFUSED	–
14	LAID (129)		FED	
15	III		–	–
16	V		–	–
17	LAID (97)		FED	
18	II		REFUSED	–
19	DIED (III)	–	–	–

Fig. 3.—Stages of *A. gambiae.*

the normal ovary development. No laboratory-bred mosquitos were used for these observations, on account of the difficulty of inducing mating in captivity.

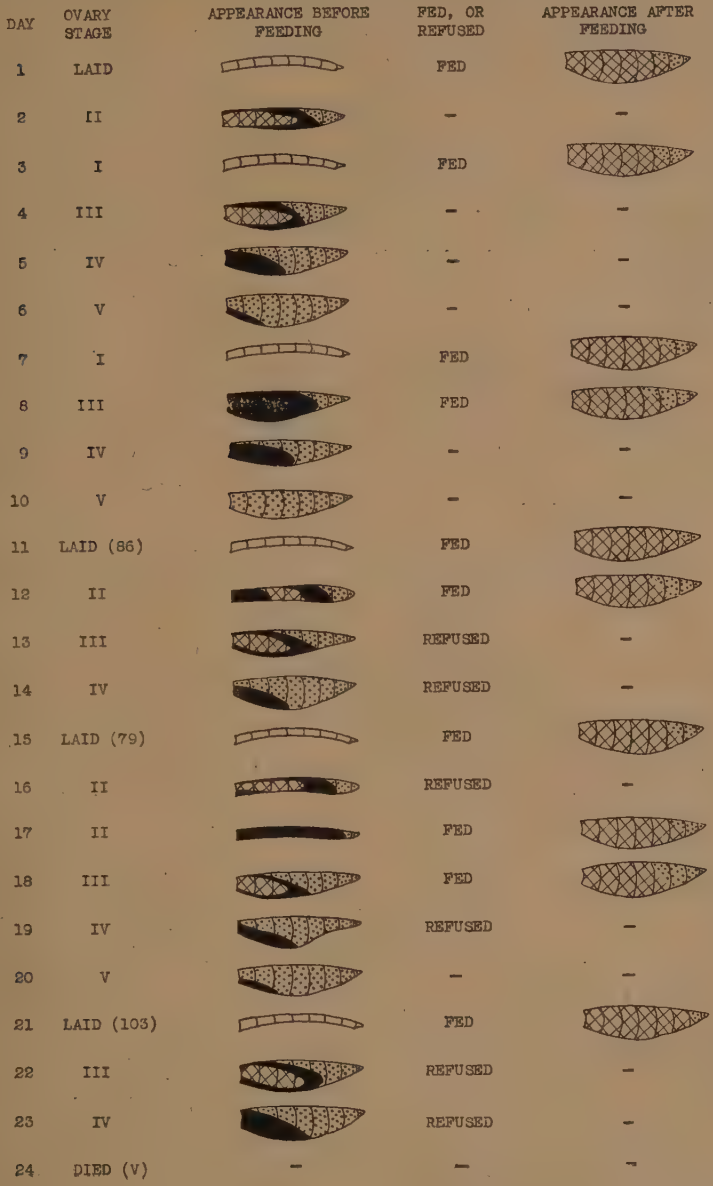

DAY
OVARY STAGE
APPEARANCE BEFORE FEEDING
FED, OR REFUSED
APPEARANCE AFTER FEEDING
1 LAID FED
2 II – –
3 I FED
4 III – –
5 IV – –
6 V – –
7 I FED
8 III FED
9 IV – –
10 V – –
11 LAID (86) FED
12 II FED
13 III REFUSED –
14 IV REFUSED –
15 LAID (79) FED
16 II REFUSED –
17 II FED
18 III FED
19 IV REFUSED –
20 V – –
21 LAID (103) FED
22 III REFUSED –
23 IV REFUSED –
24 DIED (V) – – –

Fig. 4.—Stages of *A. funestus*.
Key as for fig. 3.

In the series of diagrams of *A. funestus*, the unaccountable change between the 6th and 7th days strongly suggests that random laying may have taken place, although no eggs could be found in the cage. Several similar cases were observed which support this view, but no proof was obtained.

The rate of development of each ovary stage, based on these observations, may be summarised as follows :—

TABLE I.

Rate of development of each ovary stage.

A. gambiae Period after laying	No. of days recorded										Mean	Difference
To end of Stage II ...	1	1	1½	1½	1½	2	2	2	3	3	1¾	—
To end of Stage III ...	1	2	2	2	2	2	3	3	4	5	2½	¾
To end of Stage IV ...	2	2	2½	3	3	3	3	3	4	5	3	½
To next laying ...	3	3½	4	4	4	5	5	6	8	–	4¾	1¾
A. funestus												
To end of Stage II ...	1	1	1	1	1	1½	2½	2½			1½	--
To end of Stage III ...	1½	2	2	2	3	4	4	4			2¾	1¼
To end of Stage IV ...	2	2½	2½	2½	3½	4½	5½	5½			3½	¾
To next laying ...	3	4	4	4	4	6	6	7			4¾	1¼

These findings may be compared with those of Barber and others (1932), in Liberia, who recorded that the eggs of newly emerged *gambiae* were almost mature in 5 days.

Length of Stay in Dwellings and daytime Resting Places.

During the period 31st May, 1945, to 20th November, 1945, the Anopheline population of a small mud hut occupied by two Africans was collected by morning sprayings seventy times after a one-day interval, seventeen times after a two-day and seventeen times after a three-day interval. The catches of female *A. gambiae* and *A. funestus* were as follows : —

After 1-day interval : Total—6,512. Mean catch per day—93.

After 2-day interval : Total—1,665. Mean catch per day—98.

After 3-day interval : Total—1,532. Mean catch per day—90.

There is no significant difference between these means ; in fact, the variance of all the catches taken together is no greater than the mean of the separate variances.

From this result it follows that these mosquitos were not spending more than 24 hours in this hut, for if they were the one day catches would be lower than the others. If they spent two days, the one day catch would be half each of the others, and if they spent three days, the catches would be in the ratio 1 : 2 : 3.

Two native huts out of an isolated group of ten were then searched, first every day and then every fourth day, and the female *Anopheles* caught were separated according to the external abdominal stages, which were checked by occasional dissections to compare development of the ovaries. Results were as given in Table II.

TABLE II.

Date	Stages					Total
	I	II	III	IV	V	
			A. funestus (daily catches)			
Sept. 24 ...	2	9	33	15	35	94
Sept. 25 ...	0	22	36	10	24	92
Sept. 26 ...	10	36	36	19	25	126
Sept. 27 ...	4	38	30	16	38	126
Sept. 28 ...	8	61	30	16	45	160
Mean ...	5 (4%)	33 (28%)	33 (28%)	15 (12%)	33 (28%)	120
			A. funestus (catching every fourth day)			
Oct. 2 ...	5	49	32	29	38	153
Oct. 6 ...	3	20	32	18	33	106
Oct. 10 ...	4	47	28	12	64	155
Oct. 14 ...	5	38	26	10	47	126
Mean ...	4 (3%)	38 (28%)	30 (22%)	17 (13%)	45 (33%)	135
			A. gambiae (daily catches)			
Sept. 24 ...	0	2	6	2	9	19
Sept. 25 ...	0	1	6	1	4	12
Sept. 26 ...	0	3	6	1	3	13
Sept. 27 ...	3	1	1	0	5	10
Sept. 28 ...	0	3	4	0	6	13
Mean : ...	1 (7%)	2 (14%)	5 (36%)	1 (7%)	5 (36%)	14
			A. gambiae (catching every fourth day)			
Oct. 2 ...	0	3	6	1	6	16
Oct. 6 ...	1	1	4	5	3	14
Oct. 10 ...	0	2	3	4	10	19
Oct. 14 ...	0	2	7	0	3	12
Mean ...	0 (0%)	2 (14%)	5 (36%)	2 (14%)	5 (36%)	14

There was practically no difference between the totals or the proportions of each stage, caught after one-day and after four-day intervals. Also the numbers of each stage were maintained throughout the five daily catches, showing that female mosquitos in all stages left and entered the huts in approximately equal numbers each night, and that there was a sufficient reserve of all stages outside these two huts (either in the other eight huts or in the bush) to maintain the numbers of each stage in these two huts despite the daily removals from them.

To determine whether the mosquitos were resting only in the other huts or also in the bush, the experiment was extended in the following way. At the same time as the morning sprayings for collection of mosquitos were made in the two selected huts, as many as possible of the mosquitos in the other eight huts were killed by spraying. This was done in the group of ten huts used in the previous experiments, and also in another isolated group of about 20 huts. There was a striking difference from the results obtained when the unsearched huts were unsprayed.

The numbers of stages I and II were maintained each day (completely with *A. gambiae*, less completely with *A. funestus*), but the numbers of the later stages fell very strikingly after the first day. (See Table III.)

TABLE III.

Date	Stages					Total
	I	II	III	IV	V	
			A. gambiae			
Jan. 21, 46 ...	24	63	55	7	63	212
Jan. 22, 46 ...	14	74	26	0	6	120
Jan. 23, 46 ...	12	62	12	1	13	100
Jan. 24, 46 ...	13	69	18	3	4	107
Jan. 25, 46 ...	18	128	15	0	2	163
Jan. 26, 46 ...	12	58	7	0	5	82
Jan. 27, 46 ...	0	31	5	1	0	37
Dec. 19, 45 ...	60	86	80	29	241	496
Dec. 20, 45 ...	36	123	30	4	22	213
Dec. 21, 45 ...	76	98	26	3	7	210
Dec. 18, 45 ...	10	16	6	3	1	36
Dec. 19, 45 ...	2	19	6	0	0	27
Nov. 26, 45 ...	7	48	58	9	16	138
Nov. 27, 45 ...	0	6	12	0	2	21
Nov. 28, 45 ...	9	3	3	3	3	20
Mean 1st days	25 (11%)	53 (24%)	50 (23%)	12 (5%)	80 (36%)	221
,, other days	29 (26%)	61 (54%)	15 (13%)	1 (1%)	6 (5%)	112
			A. funestus.			
Jan. 21, 46 ...	1	5	8	0	12	26
Jan. 22, 46 ...	2	7	1	0	0	10
Jan. 23, 46 ...	5	5	1	0	1	12
Jan. 24, 46 ...	1	3	0	0	0	4
Jan. 25, 46 ...	3	12	0	0	2	17
Dec. 19, 45 ...	7	7	1	2	15	32
Dec. 20, 45 ...	2	11	1	3	2	19
Dec. 18, 45 ...	11	10	11	1	18	51
Dec. 19, 45 ...	3	10	1	0	1	15
Nov. 2, 45 ...	5	38	41	4	30	118
Nov. 3, 45 ...	12	22	4	0	1	39
Nov. 4, 45 ...	9	3	1	0	0	13
Nov. 9, 45 ...	12	6	8	10	5	41
Nov. 10, 45 ...	3	5	2	0	2	12
Nov. 26, 45 ...	14	31	16	2	44	107
Nov. 27, 45 ...	2	3	3	0	0	8
Nov. 28, 45 ...	13	2	2	3	0	20
Mean 1st days	8 (13%)	16 (26%)	14 (23%)	3 (5%)	21 (34%)	62
,, other days	5 (36%)	7 (50%)	1 (7%)	0·5 (3%)	1 (7%)	14

These results can only mean that the mosquitos in stages III, IV and V had been resting in the other huts and not in the open bush, and that after the first day the only mosquitos found in the huts in the morning (apart from a few that escaped the sprayings) were those newly emerged or newly returned from egg laying.

It was therefore concluded :—

1. That female *A. gambiae* and *A. funestus* throughout the year at Taveta did not normally spend more than 24 hours in any one hut, but moved between huts every night, whether in need of a blood-meal or not.

2. That, at any rate during the early rains (November to January), female *A. gambiae* and *A. funestus* did not rest in the open bush in the daytime, except when just emerged and just before and just after egg laying, so that nearly all the mosquitos of these species are to be found during the day in native huts.

The first conclusion is in accordance with previous work in West Africa. Gordon and his collaborators in Sierra Leone in 1932 found that both species did not normally spend more than 24 hours at a time in native houses, and Barber & others (1932) came to the same conclusion working in Liberia. On the other hand, De Meillon (1934) in the Transvaal showed that females of these species, in the wet season, enter a house within a short time of hatching and remain until the eggs are practically developed, but that, in the dry season, they cannot be found in houses, which are open to the wind and direct sunlight, during the day.

Notes on the Feeding Habits and Oviposition.

Feeding Habits.

During these observations, food was offered in the morning, and normally no difficulty was experienced in inducing the insects to feed by daylight, even, in some instances, in full sunlight. This was also found to be the case with wild mosquitos, since frequent cases of biting by both vector species were recorded, particularly between sunrise and 2.0 p.m., even in open huts of which the walls do not reach the roofs.

Whilst feeding mosquitos under observation, it was found that in many cases the proboscis would be fully inserted and withdrawn several times before actual feeding took place, each ineffective bite producing the usual weal and accompanying irritation.

A considerable number (40–50) of females of both vector species have been examined for ovary development after being allowed only one blood-meal immediately after laying. In one case only was the cycle of ovary development completed, namely that between the 14th and 17th days in the series of observations on *A. gambiae* as shown in fig. 3. In all other cases death occurred, generally within 48 hours, or at most within three days. It would thus appear that at least two blood-meals are normally required during each cycle of development. However, the fact that no wild-caught females were found to have ovaries beyond early stage III suggests that the above may not be true in nature.

A. gambiae females have been found to take a blood-meal every 24 hours if given the opportunity ; in one case, a meal was taken for 11 days in succession, during which period two complete cycles of ovary development took place. In some instances, however, daily feeding appeared to inhibit laying. A female placed under observation when apparently in Stage IV fed for six days in succession, and laid for the first time on the ninth day. During the whole of this period the external appearance before feeding was Stage V.

In *A. funestus*, daily feeding has only been found to occur for three successive days, but several cases have been observed of feeding taking place in Stage V, followed by laying within 24 hours.

Barber and Ollinger (1931) also found irregularity of feeding in captivity.

When allowed to feed undisturbed, females of both vector species invariably engorged until some blood had passed right through the body and had been ejected from the anus. In some instances, the amount so passed was estimated to be at least equal to the total volume of the distended stomach, whilst the resultant spot of blood on the skin was situated 5–6 mm. behind the distal end of the abdomen. Several smears from this ejected blood were examined microscopically, and there appeared to have been no effect either upon the coagulation properties or upon the individual corpuscles.

At the beginning of the meal the body would be held at an angle of about 45° from the surface, and gradually lowered during engorgement until the ventral surface rested on the skin. After withdrawal of the proboscis the body returned to the 45° attitude.

Digestion of Blood-meals.

Table IV indicates approximately the period required for the complete digestion of a full blood-meal. Since observations were made every 24 hours, the periods shown in the table are taken from the time of feeding to the first subsequent observation at which the stomach content was no longer visible externally. Thus when the period of digestion is shown as 72 hours, the stomach had, in fact, ceased to be visible at some time between 48 and 72 hours.

TABLE IV.

Periods of digestion of a full blood-meal.

Species	No. of records of complete digestion in :—				Mean
	24 hours	48 hours	72 hours	96 hours	
A. gambiae	3	17	3	0	48 hours
A. funestus	1	6	5	1	60 hours

Oviposition.

Egg-laying normally takes place at night, and gravid females show great reluctance to lay either by white or blue artificial light. The eggs are generally white when laid, and darken gradually, reaching their normal black condition after about two hours. Occasionally partial pigmentation may take place within the ovaries, seven such examples having been found out of a total of 63 ovary dissections.

On nine separate occasions, females of *A. gambiae* have been observed whilst ovipositing. In five instances the insect settled on the water surface, and in each case the normal resting attitude was maintained throughout, with the body at an angle of approximately 45°, the distal end of the abdomen being uppermost. No movement of the abdomen could be detected, and the eggs emerged at regular intervals, falling to the water surface to form the characteristic clusters. In one of these cases, the insect was in a restless condition on first settling, and walked slowly over the water surface for about 1½ minutes, leaving an intermittent trail of eggs. This trail was 2–2½ inches in length, and was subsequently found to comprise 63 eggs. A further 97 eggs were laid in a single cluster at the end of the row, the mosquito remaining stationary for about 3 minutes. A total of 160 eggs was thus produced in 4½ minutes.

On three other occasions, laying has been observed whilst the female rested on the sloping side of the bowl, about 1 inch above the water surface. The attitude was again normal, relative to the bowl surface, and the distal end of the abdomen did not project over the water. The eggs therefore fell on to the side of the bowl, to which a few adhered, whilst the majority rolled immediately to the water.

In one instance, the insect was restless, and settled several times both on the water and on the bowl without laying. Oviposition began whilst the female was on the water, but was frequently interrupted ; the eggs being laid in small clusters all over the bowl, both from the water surface and from the side, during a period of about 30 minutes. It appeared that considerable difficulty was experienced, since the eggs did not emerge freely, and the left hind leg was employed throughout to assist delivery. It is possible that this apparent difficulty partially accounted for the restless condition.

It was found in about 60 per cent. of cases in which laying had taken place unobserved that the egg clusters were situated well away from the sides of the bowl or from the perches provided, whilst the remainder were in contact with the edge of the water. There appears to be no indication that laying ever takes place from grass stems. Eggs were frequently found to be in lines, which suggests that laying on the move is not an unusual practice.

Oviposition in *A. funestus* was observed on three occasions ; in one case, the female rested with the fore legs on the bowl, the middle legs on the water and the hind legs in the air. The body was again in the normal resting position, and the eggs formed a compact cluster which was drawn to the side of the bowl as soon as the insect left the water. The other two females to be observed during oviposition settled on the bowl, and laid in a similar manner to *A. gambiae*. Other egg clusters of *A. funestus* have shown a rather greater proportion in contact with the edge, which suggests that in about 60-70 per cent. laying takes place from the water edge. In only one case was an egg-cluster found to be in contact with the base of a grass stem.

On several occasions, complete clutches of eggs sank, whilst others laid on the same water floated normally. In some cases, part of a single clutch sank, and the remainder floated. A considerable number of these sunk eggs were kept under observation, but in no case did hatching occur.

Under laboratory conditions laying is often interrupted, and resumed after an interval of 24 hours or more. One specimen of *A. gambiae* laid 117, 45 and 101 eggs on three successive days. Several other instances were recorded of a second clutch being laid within 48 hours of the preceding clutch. In these cases the abdomen did not revert to the characteristic Stage I condition until after the second laying.

Whenever isolated females laid, the eggs were counted in order to determine the normal clutch size. In addition, several Stage V females were dissected, and the eggs counted in the ovaries. The results of these observations are as follows :—

TABLE V.

Normal clutch size in *A. gambiae* and *A. funestus*.

A. gambiae	No. of examples	No. of eggs	Maximum clutch	Minimum clutch	Average clutch	Mean
Clutches laid	29	4,841	325	80	166·9	175
Ovaries dissected ...	26	4,778	279	127	183·7	
A. funestus.						
Clutches laid	32	3,918	181	30	122·4	123
Ovaries dissected ...	37	4,569	160	75	123·5	

It is possible that the minimum clutch laid by each species may have been an example of interrupted laying.

These observations were made towards the end of a long period of exceptionally dry conditions. The ensuing wet season was abnormally short, and only a few further observations could be made. These were considered inadequate for recording in detail, but the mean egg number of each species was found to be appreciably lower during the wet period.

The largest recorded series laid by a single *A. gambiae* amounted to a total of 917 eggs, produced in four clutches over a period of 15 days.

In the case of *A. funestus*, five fertile clutches have been laid by an isolated female under laboratory conditions, and in *A. gambiae*, four clutches. It therefore seems probable that the majority of females of both species mate only once.

Hatching.

When the egg hatches, the larger (anterior) end opens by means of a diagonal split, as shown in fig. 5.

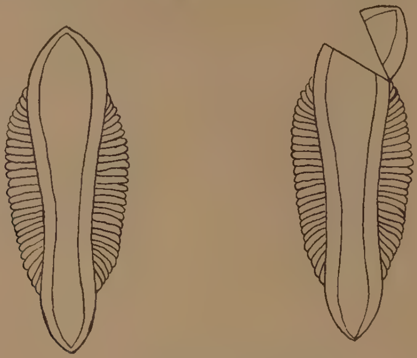


Fig. 5.—Egg opening.

The position of this split appears to be invariable. The cap is not detached, and returns to its normal position after the larva has escaped. In some cases the egg springs open sharply, causing appreciable disturbance to adjacent eggs. The emergence of the larva is extremely rapid (3–5 seconds), and is not accompanied by violent wriggling as may be the case with Culicine larvae. Immediately after emerging, the larva attains a length about 1·3 times that of the egg.

Observations on the period of development of the egg, at a mean temperature of 25·5°C., may be summarised as follows :—

TABLE VI.

Development of ova from laying to hatching.

Species	No. of records of development in :—				Mean—not more than—
	24 hours	48 hours	72 hours	96 hours	
A. gambiae	1	5	0	1	50 hours
A. funestus	0	3	1	0	54 hours

These observations were carried out entirely under laboratory conditions, and development may have been delayed in consequence.

Under field conditions Hopkins (quoted by Evans, 1938) found that the egg period of *A. funestus* at 23·4°C. was 3 days, and of *A. gambiae* between 22·4 and 24·6°C. 2 days.

Summary.

Females of both *A. gambiae* and *A. funestus* were found to enter buildings throughout the night, but more *A. gambiae* entered between 1 and 4 a.m. and more *A. funestus* just before dawn. Males of both species entered only just before dawn.

The rate of development of the ovaries was studied in individual mosquitos isolated in cages in the laboratory. The mean period between egg layings at a mean temperature of 25·5°C. was about 4¾ days in both species.

It was found that females of *A. gambiae* and *A. funestus* did not normally spend more than 24 hours in any one hut; but that they rested outside buildings during the day time only when newly emerged and just before and just after egg-laying.

Observations were made on the feeding and oviposition habits. It was found that females of both species would sometimes feed daily, whereas digestion of a full blood-meal occupied 24–72 hours in *A. gambiae* and 24–96 hours in *A. funestus*. The average dry season clutch of *A. gambiae* was 175 eggs and of *A. funestus* 123; wet season clutches appeared to be smaller.

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References.

7

Barber, M. A. & Ollinger, M. T. (1931). Studies on malaria in Southern Nigeria.—Ann. trop. Med. Parasit., **25**, pp. 461–501.

——, Rice, J. B. & Brown, J. Y. (1932). Malaria studies on the Firestone rubber plantation in Liberia, West Africa.—Amer. J. Hyg., **15**, pp. 601–633.

Covell, G. (1939). How to do a malaria survey.—Hlth Bull. no. 14 (Malar. Bur. no. 6), 4th edn., 208 pp. Delhi.

De Meillon, B. (1933). On *Anopheles funestus* and its allies in the Transvaal.—Ann. trop. Med. Parasit., **27**, pp. 83–97.

——. (1934). Observations on *Anopheles funestus* and *Anopheles gambiae* in the Transvaal.—Publ. S. Afr. Inst. med. Res., no. 32, pp. 195–248.

Evans, A. M. (1938). Mosquitoes of the Ethiopian Region. II.—Anophelini, London, Brit. Mus. (Nat. Hist.), pp. 156, 316.

Gibbins, E. G. (1933). The domestic *Anopheles* mosquitoes of Uganda.—Ann. trop. Med. Parasit., **27**, pp. 15–25.

Gordon, R. M., Hicks, E. P., Davey, T. H. & Watson, M. (1932). A study of the house-haunting Culicidae occurring in Freetown, etc.—Ann. trop. Med. Parasit., **26**, pp. 273–345.

182 Haddow, A. J. (1942). The mosquito fauna and climate of native huts at Kisumu, Kenya.—Bull. ent. Res., **33**, pp. 91–142.

Senior White, R., Ghosh, A. R. & Venkat, Rao, V. (1945). J. Malar. Inst. India, **6**, pp. 129–215.

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THE PENETRATION OF INSECT EGG-SHELLS.

II.—THE PROPERTIES AND PERMEABILITY OF SUB-CHORIAL MEMBRANES DURING DEVELOPMENT OF *RHODNIUS PROLIXUS*, STÅL.

By J. W. L. Beament, M.A., Ph.D.

Agricultural Research Council, Unit of Insect Physiology,
Department of Zoology, Cambridge.

A previous paper (Beament, 1948) describes an investigation of the penetration of liquids through the part of an insect egg-shell which is constant throughout the life of the embryo—the chorion, the cement and the primary wax layer. Ovicidal experiments were performed almost entirely on one-day-old eggs, in which the resistance of other membranes of the shell is at a minimum (see below). The changes in resistance, due to embryonic activity during incubation, will now be examined. These changes depend primarily on two factors :—

i. The formation of specific membranes inside the chorion, altering the rate at which substances enter the embryo; changes in the chorion caused by progressive chemical processes; the effects of embryonic secretions.

ii. Changes in the sensitivity of the living embryonic material.

The second factor has no satisfactory meaning. It usually expresses the residue of inexplicable phenomena at the end of an ovicidal or insecticidal study. If, for example, the changes in sub-chorial membranes had not been discovered, then variations in resistance might be attributed to alterations in the susceptibility of the *living organism,* attendant on its development. However, having found such structural alterations, one cannot necessarily attribute *all* the changes in resistance during development to these modifications. The living material inside the egg-shell may become more, or less, sensitive to any one poison as it develops. But if a group of substances—having common physico-chemical properties but different chemical constitutions—indicate similar changes in resistance, those changes are not likely to be the result of the varying sensitivity of the living material. Protoplasm will probably be killed in very different ways by, for example, acetone and by chloroform. Both these chemicals are lipophiles and have wax solvent properties. If the susceptibility of the developing egg changes in a similar manner, when a group of wax solvents are compared, it is more logical to conclude that changes in the external membranes (the non-living materials) are the cause, especially if the properties of such membranes seem to confirm ovicidal results.

At the end of this investigation it may be necessary to postulate varying embryonic susceptibility; even so, when one knows the complete mechanism by which each poison reaches its eventual site of action, the expression " change in the overall resistance of the embryo " should have no meaning at all.

Preliminary Evidence.

Many workers (Stone & LaHue, 1946; Gough, 1940; Kuenen, 1946; Speyer, 1934; etc.) have noted differences in the resistance of insect eggs at different times during their development. It is well known that eggs, such as those of the over-wintering fruit-tree pests, are more difficult to kill during diapause, than earlier or later in the season. Slifer (1937) has described the production, and later resorbtion, of membranes on the inside of the chorion of *Melanoplus differentialis*, Thos., and later (1946) she suggests that the breakdown of lipophilic material at the hydropyle of that egg may accompany the end of the diapause period.

In the *Rhodnius* egg, Beament (1946c) recorded that the transition point in the water-loss/temperature curve changes during the sixth day of incubation at 25°C. and supposed that this indicated the secretion of a further wax layer within the shell. The existence of a fertilisation membrane has also been recorded (Beament, 1946a).

It may be convenient to summarise what is already known of the entry of liquids into eggs of *Rhodnius prolixus*. Beament (1948) has shown that the chorion of the egg is virtually impermeable to ovicidal liquids and that the embryo receives its lethal dose through the micropylar tubes which pierce the whole thickness of the chorion (fig. 3). Once a liquid has traversed a micropyle, only a thin layer of wax (Beament, 1946c) and the sub-chorial membranes here considered, protect the embryo (fig. 1).

The factors involved in penetration are analysed in the following example, which serves to indicate the existence of changes in the resistance of *Rhodnius* eggs during the 15 days in which they develop. If the eggs are immersed in glacial acetic acid, cytolysis in the yolk can be observed through the shell. The acid enters rapidly and yolk fluids are not displaced at a corresponding rate. Consequently the yolk swells up and in a short time the cap is displaced, often with considerable force. When this happens, internal hydrostatic pressure must overcome the adhesion of the seal and rupture the sub-chorial membranes. The period of time before the cap is thrown off may be divided into the following intervals of time in which the liquid :—

(*a*) Traverses the micropyles.
(*b*) Penetrates the wax layer.
(*c*) Penetrates the sub-chorial membrane.
(*d*) Achieves sufficient pressure to break the seal.
(*e*) Achieves sufficient pressure to rupture the sub-chorial membranes.

Factors *a, b* and *d* are constant throughout the incubation period. Thus, differences in the recorded times for bursting represent differences in the permeability and in the mechanical strength of the internal membranes. Both indicate changes in the overall resistance of the egg. To this analysis must be added the factor that the yolk and embryo are imbibing the acid. As the yolk is converted to embryonic material, a gradual increase, or decrease in the imbibing force might be expected. However, no such gradual change was observed.

The bursting times for eggs at different stages of development are as follows :—

1-day-old eggs	9·7 minutes
4-day-old eggs	15·3 minutes
8-day-old eggs	18·6 minutes
12-day-old eggs	19·1 minutes
14-day-old eggs	11·0 minutes

The figures represent the average of the individual values for 20 eggs obtained from medium-aged females. Sterile and one-day-old eggs burst very rapidly; after four days' incubation, the eggs are much more "resistant." Although eight-day-old eggs require longer periods of immersion, the rate of increase is not maintained and there is no change between the values for eight- and for twelve-day-old specimens. But fully 24 hours before normal eclosion, the egg bursts in a period very little longer than that required by the one-day-old group. During the period eight to twelve days considerable embryonic growth takes place (Mellanby, 1935 & 1936). By 14 days the membranes appear to have reverted to their original properties.

Further evidence for changes in the sub-chorial membranes was obtained during investigations on the action of wax solvents (Beament, 1948). Eggs were desiccated after being submerged for various intervals in lipophilic solvents. After relatively short periods of immersion, desiccation produced the collapse of the eggs with embryos at the earliest and latest stages of development. At intermediate stages of embryonic formation, much longer treatment with wax solvents was needed. Some of the controls to these experiments consisted of eggs of similar stages of development which had been killed by fumigation with hydrogen cyanide. Again, when the embryo was partially developed, the egg collapsed more slowly than at the start or end of the incubation period.

This evidence points to considerable changes in the sub-chorial membranes lying inside the primary wax layer but separate from the living embryonic material. An investigation of the properties and of the physiological consequences of such membranes was therefore undertaken.

Material.

Beament (1948) outlines those properties of *Rhodnius* eggs which make them suitable material for investigations of this nature. Briefly, adults and eggs are incubated at 25°C. and 60-70 per cent. relative humidity. Eggs are removed from culture jars each morning and kept separately. The embryos develop very uniformly under these conditions, hatching on the sixteenth day after oviposition; 15 age groups are thus available which are more uniform since the egg is fertilised immediately before it is laid (Beament, 1946b). In observing some 2,000 eggs, less than 1 per cent. were infertile and less than a quarter of 1 per cent. of the viable eggs failed to hatch at 25°C. and 70 per cent. humidity; these figures relate to the eggs from females up to four months old. Young females deposit much more of a cement over the surface of their eggs which often occludes the micropylar openings, gives the eggs greater resistance to ovicides, and makes replication of such tests more variable (see Beament, 1948). Hence, in the experiments outlined here, eggs from females two to four months old were used being the most uniform of the available material.

The Fertilisation Membrane.

Beament (1946a) has recorded the existence of a membrane in developing *Rhodnius* eggs attached to the inside of the primary wax layer (fig. 1, *b*). The formation of this membrane was demonstrated in the following way.

Eggs, obtained before and after oviposition (and thus before and after fertilisation), were immersed in Ringer's solution and the yolk washed out through a hole cut in the rear end of the shell. Great care was taken to wash out the whole of the thick vitelline membrane; the operation was carried out very quickly. The empty shells were dried, filled with a saturated aqueous solution of borax carmine and again dried in a desiccator. This was repeated to ensure a heavy deposit of pigment on the inside of the shell. The shells were immersed in molten paraffin wax, care being taken that the shell cavities were also filled. After cooling, the shells were cut into halves along their longitudinal axes; the pieces of wax, each with a half shell embedded in one face, were observed under a binocular microscope whilst immersed in xylene.

When waterproof eggs, taken from the ovary (before fertilisation), were examined, the central plug of embedding wax dissolved away and the dry dye crumbled off the inside of the shell due to the solution of the primary wax layer which was the substrate of the deposited stain (fig. 1, *a*). Similar results were obtained with sterile eggs, if examined within a few hours after they had been laid by virgin females. But preparations made as soon as ten minutes after eggs had been laid by fertilised females behaved very differently. In these eggs, when the wax plug had dissolved, the stain came away in a continuous sheet supported by a thin membrane. This membrane is a true *fertilisation membrane.* It covered the whole of the inner surface of the shell and cap and was recessed into the inner portion of the seal, where it covered the micropyles (fig. 1, *b*).

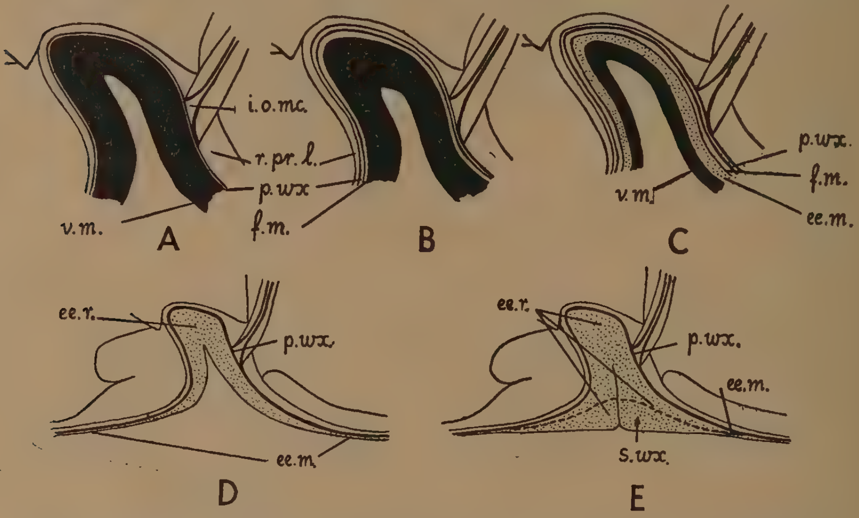

Fig. 1.—Changes in the sub-chorial membranes during incubation. Longitudinal sections of the inner portion of the junction between shell and cap, passing through micropyle (*i.o.mc.*). (*a*) Before fertilisation; primary wax layer (*p.wx.*) attached to the resistant protein layer (*r.pr.l.*) with vitelline membrane (*v.m.*) in inner recess. (*b*) Immediately after fertilisation and oviposition the wax layer is supported by the fertilisation membrane (*f.m.*). (*c*) Twenty-four hours after oviposition; material (*ee.m.*) is being added to the fertilisation membrane. (*d*) Three days after oviposition; the thickened epembryonic ring (*ee.r.*) is forming. (*e*) The sixth to twelfth days of incubation; the recess is filled by the epembryonic ring, the membrane (*ee.m.*) being much thinner. The inner surface is impregnated with secondary wax (*s.wx.*). The dotted line delineates the material which is broken down between the thirteenth day and hatching.

The membrane is exceedingly thin when first formed; it would appear that material is slowly added to it from its inception. About 30 minutes after fertilisation the membrane is probably less than 0·3 micron thick, but it is rigid and considering its thickness it is remarkably tough.

The membrane of the one-day-old egg was colourless and almost transparent; it is stained palely in neutral red, basic and acid fuchsin, in aqueous and more particularly in alcoholic media. Safranine at a *p*H of 11·5 stained it much more heavily than Orange G at a *p*H of 3 (Fraenkel-Courat & Cooper, 1944). It was coloured by iodine and tanned to dark brown by cold *p*-benzoquinone. There was a strong polyphenol reaction with ammoniacal silver nitrate (Lison, 1936), but the colour produced by warm Millon's reagent was pale brown rather than pink. There was also a strong positive xanthoproteic reaction. No staining could be detected after immersion in picric acid and the ninhydrine reaction was negative.

It would be difficult to detect faint colourations of a membrane so thin and diaphanous as the fertilisation membrane soon after its formation. These tests were carried out on membranes which adhered to the inside of fragments of shell and were afterwards removed for examination. The reactions did not differ if, first, the membrane had been removed by cold, or by boiling chloroform. The membrane was resistant to cold potash and to nitric acid but was broken down by nitric acid saturated with potassium chlorate. It was dissolved by diaphonal and did not give the " chitosan " test (Campbell, 1929). A number of methods for detecting pore canals were applied to the membrane; Rapid golgi method, drying in air (Wigglesworth, 1933); and silver bromide method (Beament, 1946b), but no pores could be demonstrated.

Polyphenols are scattered throughout the membrane. In Arthropod membranes so far examined (Wigglesworth, 1945, 1947; Lees, 1947; Lees & Beament, 1948; Beament, 1946c); polyphenols are often associated with organised wax layers and are present in the substrate on to which wax is deposited. The primary wax layer of the *Rhodnius* egg has been laid down on the inner polyphenol layer of the chorion before the fertilisation membrane is formed. It must be concluded that the polyphenols in the fertilisation membrane help its firm attachment to the wax surface.

The membrane would appear to be composed of protein and its resistance to solution suggests that tanning or other hardening processes have been active. The membrane is not attacked by trypsin or pepsin solution but it is softened and, to some extent, dissolved by solutions of thioglycolic acid at a *p*H of 12 (Goddard & Michaelis, 1934). It may, therefore, be suggested that the fertilisation membrane owes some of its resistance to cystine bonds in the protein chains and is similar to the protein of the endochorion in this respect.

Permeability of Membranes.

A large number of experiments were performed, using eggs of each stage of embryonic development. After treatment with liquids (particularly with wax solvents) eggs were subjected to osmotic forces and transferred to stains and other solutions. At the end of each experiment, eggs were examined under a binocular microscope; they were cut open in oil, glycerine or water and the yolk, shell and extra-embryonic membranes were examined. The shell may collapse in a hypertonic solution, or alternatively, osmosis may deflate the yolk inside a detached fertilisation membrane, without deforming the chorion. If, on the other hand, the immersant is hypotonic to the egg, it may burst, but before this happens the cap becomes markedly conical; if the egg is more than four days old its lateral dimple may be expressed.

The following solutions were used in penetration tests :—

Distilled water and Ringer's solution.
Water saturated with sodium chloride at 25°C.
Water saturated with glucose at 25°C.
Basic fuchsin dissolved in water and in Ringer's solution.
p-benzoquinone, in Ringer's solution.
Picric acid, in Ringer's solution.
Basic fuchsin in 70 per cent. alcohol.
Sudan III in 70 per cent. alcohol.

Eggs appeared to be quite unchanged if immersed in any of these solutions for 24 hours regardless of the stage of embryonic development. Solutions were made up in Ringer's solution because eggs de-waterproofed by wax solvents may burst in hypotonic liquids and no evidence of penetration can be obtained from them. In addition, 1 per cent. potassium permanganate solutions in Ringer's fluid have no effect for some 12 hours but after longer periods of immersion the shell becomes soft and it may crack or burst. Other solutions, including emulsifiers, wax solvents and dyes with various molecular sizes were also used to augment the information from the above selection of indicators.

One-day-old eggs.

Batches of one-day-old eggs were immersed in various liquids and numbers were transferred to each of the above solutions. After treatment with chloroform (1-4 hours), benzene (2-6 hours), xylene (2-6 hours) the egg swelled in distilled water and the shell collapsed in salt and sugar solutions. Potassium permanganate solutions did not enter the yolk. Thus, after part of the wax layer has been removed, the fertilisation membrane is semi-permeable and does not allow uni-atomic ions to pass through freely. The membrane was also impermeable to picric acid and to basic fuchsin in Ringer's solution but permeable to *p*-benzoquinone. This last-named chemical must traverse the membrane by solution in the membrane substance for the membrane was stained by it. (As all these liquids pass to and from the yolk cavity *via* the micropyles, the phenomena recorded are characters of the sub-chorial membranes and not of the chorion (see Beament, 1948; and fig. 3).)

On the other hand, Sudan III and basic fuchsin in 70 per cent. alcohol both entered and stained the yolk. Sudan III emulsified in water, using the ester C.09993, did not do so. After longer application of wax solvents, eggs which had not burst were subjected to similar treatments. The results differed only in that shells did not collapse in very hypertonic solutions. The yolk shrank inside the fertilisation membrane which had been split off when the primary wax layer was dissolved.

After treatment with absolute ethyl alcohol for over four hours, eggs swelled in distilled water but did not collapse in salt or sugar solutions. Potassium permanganate passed into the yolk freely: so did Sudan III and basic fuchsin in 70 per cent. alcohol and *p*-benzoquinone in Ringer's fluid. Basic fuchsin in Ringer's solution and emulsified Sudan III in water did not penetrate, but picric acid would colour the yolk.

From these experiments it must be concluded that absolute ethyl alcohol modifies the fertilisation membrane, having passed through or dissolved the primary wax layer. The " pore " size of the membrane has been increased so that small particles, such as the ions of sodium chloride and potassium permanganate penetrate freely; yet it is still a barrier to the large particles of the yolk, for the egg swells in water. The " pores " in the membrane, produced by alcohol, must be larger than the glucose and rosanaline (basic fuchsin) molecules and neutral red will also enter the yolk. This still does not explain the discrepancy between alcoholic and aqueous solutions of fuchsin and Sudan III but a continuous medium of alcohol may be necessary before these dyes enter the yolk in sufficient quantities to be detected. Fuchsin is not at all soluble in Ringer's solution and very little Sudan III can be emulsified. The apparent changes in " pore size " cannot be explained by the differential action of the primary immersant on the wax layer; chloroform and the other liquids used above are much more efficient wax solvents than alcohol.

Further investigation entailed the use of various concentrations of ethyl alcohol and of members of the aliphatic alcohol series. Using ethyl alcohol of concentrations up to and including 70 per cent. in water, eggs did not behave any differently from normal untreated eggs. This is, of course, explained by the fact that lower concentrations of alcohol cannot dissolve the wax and reach the membrane. After eggs had been submerged in 90 per cent. alcohol for 24 hours, they swelled in distilled water but flattened in sugar and very slightly in salt. Potassium permanganate and *p*-benzoquinone gave doubtful reactions; they probably penetrated very slowly. Neither basic fuchsin nor Sudan III in 70 per cent. alcohol entered the yolk. Obviously here large particles cannot reach the yolk because the wax layer has been dispersed but little.

On the other hand if the wax was first removed by submerging eggs in chloroform for four hours, before transferring them to solutions of alcohol in water, the size of the apparent " pores " in the membrane was increased. The final effect might be proportional to the strength of alcohol employed and the length of time during which the eggs are immersed in it. Seventy per cent. alcohol produced " pores " and 90 per cent. solutions gave almost the same effect (after chloroform) as the absolute liquid alone.

Methyl alcohol did not increase the " pore size " of the membrane to anything like the same extent, even after chloroform had removed the wax layer. But absolute propyl and butyl alcohols were quite effective and iso-propyl and ter-butyl alcohols are perhaps even more efficient than the ethyl homologue. The general effect of alcohols might conceivably be connected with the occurrence of the CH_3-CH_2-OH configuration. Picric acid, glacial acetic or formic acid, applied for a very short period, appeared to obliterate the effects of alcohol. In particular, picric acid, used after alcohol, prevented sugar and larger molecules from diffusing into the yolk. Salt and smaller ions continued to permeate freely.

In further experiments, wax solvents, or emulsifiers in aqueous solutions, were injected into the inside of shells prepared from one-day-old eggs by washing out the yolk. The shells were examined to see if such substances would reach the wax layer. More than one injection was needed to loosen the membrane but it was obvious that solvents, such as chloroform, acetone, benzene, etc., would traverse it. After 24 hours the large-moleculed polyethylene glycol emulsifiers penetrated sufficiently to soften the wax.

Conclusions.

When first formed the fertilisation membrane is semi-permeable to salts and larger water soluble particles. It is more permeable to lipophilic liquids but not to larger oil soluble molecules. There is no indication that large molecules may not pass through it very slowly. Alcohols have the unusual property of removing semi-permeability and making the membrane more permeable.

The fertilisation membrane does not confer any great measure of resistance on the embryo. It will, of course, satisfy the principal function of all such membranes—to prevent sperm from invading the zygote after the initial one has entered the ovum (Loeb, 1913). As the egg ages, the membrane changes (see below) and it might then protect the primary wax layer from embryonic secretions. Beament (1946c) has suggested that a hole in the primary wax layer made by the spermatozoön is repaired very shortly afterwards. The membrane is comparatively permeable when first formed and such repair activity could take place through it. Sterile eggs have no similar membrane for the first 24 hours after oviposition but if they are incubated for some days an almost identical membrane is eventually produced. This may indicate the very first stage of parthenogenetic development in the egg.

The membrane—up to six days' incubation.

When eggs are incubated at 25°C., and 60 per cent. relative humidity, material is added to the fertilisation membrane during the first five to six days. Over the unspecialised part of the shell, the membrane may become as much as 0·5 microns thick, but it is much thinner on the inner surface of the cap. In the inner recess of the micropylar region (fig. 1, *c*, *d*), the fertilisation membrane follows the contour of the shell (see above); only at this point does the yolk cavity depart from a smooth elipsoidal shape. However, material is added to the membrane at a much greater rate in the recess opposite the inner micropylar openings. The recess is slowly filled up and after five days' incubation, the shell cavity is uniformly smooth. The membrane may be as much as 10 to 15 microns at its maximum thickness (fig. 1, *e*).

The name proposed for this material is the *epembryonic membrane*. It lies outside the normal extra-embryonic membranes, such as the amnion and is a non-living structure derived from the embryo; it is not part of the chorion. Although (see below) much of its material is derived from the serosa, this is not a discrete product of the serosal layer and the term, serosal membrane, would be imperfect and may imply homology with the serosal cuticles of the *Melanoplus* egg (Slifer, 1937) and of the *Dytiscus* egg, etc. Though this material, together with the primary wax layer, the fertilisation membrane, and with other material added later, can justifiably be called the " sub-chorial membrane ", this part of the system, which is separated from the chorion by two layers could not be called the " sub-chorial membrane ". The thickened portion in the recess will be referred to as the *epembryonic ring*. It is obvious (see below) that this ring might give considerable protection to the embryo against invasion from the micropyles.

Sections of developing eggs show that the maximum secretion of the epembryonic membrane corresponds to the maximum development of the serosa. Mellanby (1935) has referred to enlarged cells in the anterior region of the serosal layer in *Rhodnius* eggs and these correspond to the production of the epembryonic ring. One can assume that the epembryonic membrane is chiefly a product of the serosal cells, although some changes occur before the serosa has been differentiated.

Properties of the epembryonic membrane.

During the secretion of the epembryonic membrane it is likely that the original fabric of the fertilisation membrane will be modified by chemical secretions. At all events, the two layers become one entity which cannot be resolved. Tests for protein, chitin, etc., in the membrane were repeated at intervals during incubation, but there were no specific changes in the results. Changes in the intensity of colour reactions cannot be compared in such thin membranes, as the colours may be due to components of the fertilisation membrane. On the whole, the reactions listed on p. 470 became somewhat fainter. The membrane became more resistant to solution in acids and alkali which might indicate that development produced a greater degree of tanning or vulcanisation in the protein components of the membranes. In particular, thioglycolic acid (Goddard & Michaelis, 1934) detached the membrane from the wax layer without dissolving any appreciable amount; it may possibly be attacking an unmodified portion of the fertilisation membrane material.

Just before eclosion, the membrane returns to its original properties in the one-day-old egg (see p. 479). This is due to embryonic activity; it is at least certain that the changes occurring during incubation and attendant on the production of the epembryonic membrane are reversible. On the other hand, the epembryonic ring can be obtained as a thickened solenoid of membrane and is sufficiently extensive to allow more detailed examination. This is a specialised structure and it would not be legitimate to assume that the properties of the ring are necessarily those of the very thin lamina added to the fertilisation membrane, but evidence presented below suggests that the added material is similar over the whole shell.

The ring is colourless and hyaline; it does not give any of the normal reactions for proteins (p. 470) but it does assume a pale brown oclour with *p*-benzoquinone. It does not give a polyphenol reaction and is extremely resistant to acids and alkali but is slowly attacked by " chlorated " nitric acid and by diaphenol. The " chitosan " test indicates that no chitin is present and no oil could be identified when four-day-old membranes were broken down. It might be suggested that this membrane consists of " vulcanised " proteins (similar in some respects to the proteins of the chorion (Beament, 1946b), or that it is some form of reticulin (Dempsey, private communication). Neither the membrane nor the ring contain any form of pore canal or other structure.

Changes in permeability.

While it is difficult to interpret the chemical composition of the epembryonic material, there are considerable changes in permeability. The series of immersion experiments (p. 471) were carried out on eggs of two to six days' incubation, but before considering these results, the structure of the membrane must be viewed in the light of the findings of Beament (1948). If an egg is immersed in an oleophilic liquid for a short period of time, the fluid passes down the micropyle, traverses the wax layer and must then pass through the epembryonic ring. The properties of this material do not suggest that it has a great lipophilic affinity; since the ring is relatively thick, a much longer time will be required before molecules of the oleophile reach the living material of the embryo. Ovicidal experiments (p. 482) confirm this theory that the duration of immersion for a lethal dose is increased. But while the liquid is traversing the ring it will also be dissolving, or migrating along, the primary wax layer towards the main shell and towards the inner surface of the cap. In either direction the ring becomes thinner and it will not be very long before such liquid can migrate from the wax through the much thinner epembryonic layer (fig. 3). Ovicidal experiments merely indicate the length of time which must elapse before a toxic dose of the liquid has entered the embryo; any difference in the resistance of eggs of various ages will probably embrace both modes of entry. In permeability experiments, however, eggs can be left in wax solvents for various times and subsequent immersion in tracer solutions will show whether the ring or the membrane have most resistance to permeation.

After treatment with wax solvents (chloroform, xylene, benzene, etc.), the eggs, and thus the membranes, remained semi-permeable, however long the eggs were immersed, providing the time was sufficiently long to break the wax layer at all. The rate of diffusion of small particles, such as the ions of salts, must be extremely slow, both through the ring and the membrane. Once eggs are more than three days old, fuchsin in 70 per cent. alcohol no longer enters the yolk. This indicated some change in the " alcohol " effect on pore size. The immersions in alcohols were repeated; there was little change in permeability due to the action of ethyl alcohol either alone or after wax solvents up to two days' incubation. But eggs three-days-old were semi-permeable to sugar solutions and swelled in water after alcohol treatment for 24 hours. They were still freely permeable to sodium chloride, potassium permanganate and *p*-benzoquinone in Ringer's solution. Picric acid in Ringer's solution no longer entered the egg and after applying it the membrane was semi-permeable, even in solutions of sodium chloride. It might appear that after alcohol treatment the pores in the membrane are about the size of a benzene ring. But Sudan III in 70 per cent. alcohol entered the yolk.

As the age of the egg increased, the effect of alcohol diminished rapidly. By six days, when the ring reaches its maximum thickness, the membrane was semi-permeable in sodium chloride solutions after six hours in chloroform, followed by 24 hours in absolute ethyl alcohol. Ninety per cent. alcohol had almost no effect on three-day-old eggs and the action of the alcohol homologues decreased in similar proportion.

It was concluded from the results of all permeability experiments that by the time the eggs have been incubated for six days the epembryonic ring and membrane are considerable barriers to the penetration of both lipophilic and hydrophilic particles, especially to larger molecules. Injection of solvents or emulsifiers into empty shells was not as effective in removing the membrane.

Secondary waterproofing material.

Throughout the first six days of incubation the transition point of the egg (the temperature at which desiccation increases abruptly; Wigglesworth 1945, Beament 1945) remains constant at 42·5°C. (Beament, 1946c; and fig. 2). During this time the egg loses water very slowly in an atmosphere of 60 per cent. relative humidity at 25°C. and by the fifth day there is an obvious dimple in the side of the unspecialised shell. If eggs are killed by cyanide when one day old, there is no greater rate of water loss over the first 48 hours than that of living eggs.

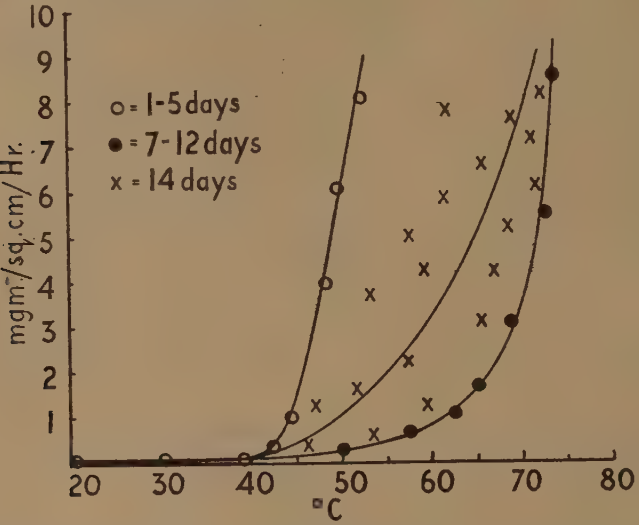

Fig. 2.—Graph of the loss of weight of single eggs when desiccated at various temperatures, showing the sharp "transition point" in eggs 1–5 days (primary wax layer); the much smoother curve of eggs 7–12 days with transition at a much higher temperature (primary wax+secondary wax) and points (×) obtained with 14-day-old eggs when the secondary wax is being emulsified.

If, on the other hand, five-day-old eggs are killed, they collapse more rapidly than living eggs, or eggs killed at eight to nine days. Beament (1946c) suggested that on the sixth-seventh day of incubation a *secondary wax layer* is produced on the inside of the epembryonic membrane which prevents the egg from drying up. The evidence for a second wax layer was based on :—

(*a*) The transition point of the whole egg rises steeply to 68°C. by the seventh day (fig. 2).

(*b*) The wax extracted from vacated shells and spread on membranes will not reproduce transition points at either 42·5°C. or at 68°C.

(*c*) Materials in solution, such as *p*-benzoquinone, placed on the inside of the shell do not stain the epembryonic layer of older eggs.

(*d*) The total wax extracted from vacated shells would approximate to a layer nearly 0·5 micron thick which, concentrated into the primary wax layer, should be obvious in sections cut without the use of wax solvents; the primary wax layer cannot be detected microscopically.

These facts certainly indicate that waxy material, extra to the primary wax layer, is added during the sixth day of incubation. But further investigation has shown that the hypothesis of a secondary wax *layer* will not explain much of the evidence. The water-loss/temperature curve obtained after seven days' incubation does not contain a sharp transition point. This would be explained by the presence of two wax layers, one of which is on the inside of the epembryonic membrane. (The characteristics of two wax layers have been described by Wigglesworth (1945) in the work of the blowfly puparium; this has a puparial wax layer and a pupal wax layer within it.) But just before eclosion (see p. 479) the inner membranes of the shell are broken down by the embryo. If the additional waxy material were arranged as a *layer* then as soon as some part of it is interrupted, the transition point should fall back to 42·5°C. In actual fact the transition point shows curious irregularities in eggs over twelve days old and drops towards 42·5°C., though it does not attain that figure by the time the egg hatches (fig. 2).

To separate the epembryonic membrane from the chorion a fragment of shell must be immersed in some wax solvent or emulsifier, whereupon both primary and secondary waxy materials will be attacked. Whatever the distribution of the secondary waxy material, the penetration of solvents from the outside of the egg will involve the disturbance of the inner wax when the primary wax layer is destroyed. But if the inner waxy material is arranged in a layer, it should be possible to remove or interrupt it by abrasion with an inert dust (Wigglesworth, 1945) just as the primary wax layer can be broken before fertilisation (Beament, 1946c). The yolk and embryos were washed from eight-day-old eggs and the empty shells were dried and cut into halves; a wad of cotton wool covered with alumina dust was drawn gently over the inner surfaces. Solutions, such as *p*-benzoquinone, which were known to colour the epembryonic membrane to some extent, were placed in the yolk cavity and when the liquid had dried the membranes were obtained by placing parts of the shells in xylene. The membranes were washed thoroughly in water and examined, but no staining of any kind could be distinguished, though it must be admitted that such experiments are very difficult where the total reaction is weak and there might be some deposit of stain left on the inside of the very thin membrane. If the secondary wax is present as a layer on the inside of the membrane, however, staining from the cut edge should take place and might be detected by contrasting it with the unstained central portion of the fragment. The epembryonic membrane will stain without abrasion before the egg is six days old.

The changes which take place at six days can be explained satisfactorily if one assumes that the secondary waxy material produced by the embryo *impregnates* the epembryonic membrane throughout most of its thickness. Since extraction in chloroform restores the membrane to the state found in five-day-old eggs, this lipoid is not chemically attached. (Compare, for example, the lipoid in the various lipoprotein layers of the shell; Beament, 1946b.) The changes in the transition temperature of the whole egg coincides with this change in the staining properties of the membranes; if eggs are killed by cyanide, neither the rate of loss of water, nor the transition temperature are affected so that the phenomenon cannot be due to the development of an active water-conserving system. At the sixth day of incubation, the embryo is just previous to blastokinesis (and see Mellanby, 1935; Tuft, private communication). The changes in transition point cannot be attributed to the formation of a larval cuticle since the larval skin of the embryo does not appear to be waterproofed at all. The transition point of the wax forming the secondary material might be in the region of 65°C. It must be present as a free wax (fig. 1, *e*) in the presence of a " 42·5°C. " wax layer. This would be an extremely hard wax for an insect product (Beament, 1945), well above the characteristic of the nymphal wax of *Rhodnius* (57·5°) which is itself high. On the other hand the wax may be less mobile when surrounded by protein molecules and the behaviour of wax in an impregnation system may not be comparable with an organised wax layer.

The series of permeability experiments were applied to eggs from the sixth to the twelfth day of incubation. After the eggs had been immersed for long periods in efficient wax solvents the results did not differ materially from those obtained before six days. The deposition of the wax has not produced any simultaneous change in the meshwork of the membrane.

Seven days to eclosion.

Until the twelfth day of incubation no change in transition temperature, rate of water-loss or permeability could be detected. Sections of the shell do not reveal any changes in the size or composition of the epembryonic membrane. The embryo continues to develop, enclosed in membranes which give it a maximum protection against desiccation. Blastokinesis takes place and the embryo grows rapidly until it occupies the whole of the shell.

When incubated at 25°C. and 70 per cent. relative humidity, the *Rhodnius* egg usually hatches during the 15-16th day of incubation. On the 13th day the embryo apparently makes first preparations for rupturing the membranes at eclosion. Water-loss/temperature curves become uneven; the transition point varies and tends towards the pre-six day figures of 42·5°C., but it is always below the maxima of the 6-12 day period (fig. 2). A determination of the transition point at each specific age involves killing eggs; there was obviously considerable variation in the time and extent of the changes in different eggs. The results indicate the disorganisation or removal of the secondary wax, though if that wax were present as a discrete layer on the inside of the membrane, any disturbance in it should result in a sudden drop to the original transition point of 42·5°C.

Microscopical examination of sections and fragments of shell indicated partial solution of the epembryonic membrane, though it does not appear to return to the dimensions of the thin fertilisation membrane (p. 470). The epembryonic ring is not completely broken down and membranes removed from the shell after eclosion have quite considerable thickenings in this region (fig. 1, *e*). It seems that, in preparation for hatching, the embryo is producing a " hatching enzyme " to soften the epembryonic membrane which must eventually be ruptured. Slifer (1937) has described the action of a hatching enzyme in eggs of *Melanoplus*. In the *Rhodnius* egg it seems likely that if the material of the epembryonic membrane is impregnated with wax, this wax must be removed in order that other enzymatic material may attack the protein.

On the day before the egg hatches, the embryonic material is completely surrounded by a clear fluid. If the cap of such an egg is prised off carefully and the shell squeezed gently, taking very great care not to rupture the embryonic integument, a drop of this fluid can be expressed onto a glass slide and examined. The liquid dried to a glassy deposit which rapidly dispersed on adding water. This process could be repeated indefinitely. The dry material was not wetted by chloroform or other wax solvents and exposing the residue did not alter its dispersion in water. But if the fluid was added to a drop of absolute ethyl alcohol there was an immediate white precipitate and waxy material was deposited at the outside edge of the droplet. This deposit did not disperse in water but was at once dissolved by chloroform and other wax solvents. Similarly, waxy deposits were produced after heating the fluid and after adding glacial acetic acid. These deposits then dissolved in wax solvents and were not wetted by water. The evidence suggests that the fluid bathing the embryo is a wax-protein complex in water. Denaturing the protein by alcohol, acid or heat causes the deposition of a wax.

The fluid gave a strong xanthoproteic reaction and a ninhydrine colouration and a positive test for argenine. Millon's reaction was negative and it was not possible to draw a conclusion from the biuret test. It seems very likely that the other component in the liquid is proteinaceous. Before denaturing processes, the edge of a fluid droplet will take up red Sudan III or black Sudan B from a solution in 70 per cent. alcohol, though this will not occur after heating. Insect and egg-shell waxes do not absorb the Sudan stains from alcohol as a rule. On the other hand such dyes can be taken up by the more lipophilic wax emulsifying materials.

It was concluded that the fluid surrounding the *Rhodnius* embryo on the fourteenth day of incubation contained waxy material derived from the secondary wax. The liquid also contains substances which are attacking the proteinaceous fabric of the epembryonic membrane and the breakdown products of this activity. A complex of protein and wax dispersed in an aqueous medium is also found in Géné's organ of the tick, *Ornithodoros moubata* (Lees & Beament, 1948). The organ applies the layer of waterproofing material to the tick egg-shell and the properties of the fluid applied to the egg are almost identical to those described above for the liquid surrounding the *Rhodnius* embryo.

Permeability changes before hatching.

Changes in the permeability of the epembryonic membrane start again at about the twelfth day of incubation; from then until eclosion, the period of immersion

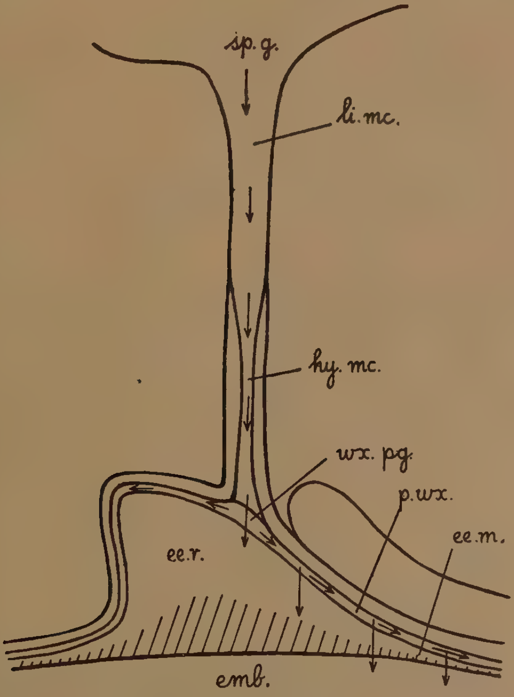

Fig. 3.—Diagrammatic section through a micropyle of an eight-day-old egg, showing the path of entry of lipophilic liquids. From the spermatic groove (*sp.g.*) liquid traverses the lipophilic part of the micropyle (*li.mc.*) and the hydrophilic portion (*hy.mc.*). It then dissolves or otherwise penetrates the wax at the base of the tube (*wx.pg.*) diffusing into the epembryonic ring (*ee.r.*) at various points from the primary wax layer (*p.wx.*), probably making its entry into the embryo (*emb.*) through the thin epembryonic membrane (*ee.m.*) which is impregnated with secondary wax (hatched area).

in glacial acetic acid sufficient to cause bursting decreases progressively. From immersion experiments it was found that solutions entered the yolk-cavity more readily; that after wax solvents had removed the waxy materials the membrane was semi-permeable to salt ions. But after the action of absolute alcohol, the pore size was increased and the egg no longer collapsed in saturated sodium chloride solutions, while potassium permanganate entered the yolk space rapidly. The egg shrank in solutions of sugar, showing that the alcohol effect was not as pronounced as it was in early eggs. The breakdown processes prior to hatching do not restore the membrane to its initial state but remove some of the embryo's protective apparatus.

When hatching takes place (Sikes & Wigglesworth, 1931) it appears from experiments in which eggs were immersed in coloured liquids and observed during the embryonic pulsations that the membranes are ruptured at the same time as the cap is displaced. Hatching fluids do not detach the membranes from the inside of the shell and the primary wax layer does not appear to be modified from its state at oviposition.

Summary of the Changes in the Sub-Chorial Membranes (fig. 1).

Time (days)	Membrane	Embryo
1–4	1° wax layer	Pre-fertilisation
0	Fertilisation membrane	Fertilisation.
2	Epembryonic material added	—
5	Epembryonic material completing; ring maximum	Serosal maximum.
6	2° wax impregnation	Blastokinesis.
6–12	No change	—
13	Emulsion of 2° wax	—
14	Partial breakdown of epembryonic membranes	—
15+	Split	Eclosion.

Throughout life the embryo is enclosed in a semi-permeable membrane which will be penetrated very slowly by most substances. When the embryo is one-day old any substance reaching it from the outer side must pass through this membrane and a wax layer. As the embryonic secretions are added penetrants have to pass through, certainly a greater thickness of semi-permeable membrane; either a fertilisation membrane with greater "rigidity" such that alcohol will no longer disrupt it, or an added lamina which is not altered by alcohol. Desiccation experiments show that, after equivalent periods of immersion in wax solvents, the four-day-old egg collapses more slowly than the one-day-old specimen. The embryo's secretions may even be somewhat resistant to the passage of water. Lipophilic liquids, penetrating the chorion, enter the sub-chorial material from the inner end of the micropyle. They either pass through the epembryonic block, which increases rapidly in the first five days of incubation, or migrate along the primary wax layer to the thinner membrane and penetrate from there (fig. 3). Both these mechanisms of entry require longer time intervals before the living material is reached, compared with the one-day-old egg. Hydrophiles normally reach the embryo by migrating along the endochorion protein layer (Beament, 1948) so that the presence of the epembryonic material may not have so much effect on their entry, but some portion of the inner recess region may be precluded from participating in the diffusion (fig. 4). In general, the deposition of the epembryonic material will make eggs more resistant but may have most effect on lipophilic liquids.

When the epembryonic material is impregnated with wax at six to seven days, the embryo should obviously be provided with very much greater protection against hydrophilic penetrants. But this secondary wax need not necessarily make the passage of lipophiles more difficult. By analogy with the work of Webb & Green (1945) and Hurst (1943), it is probable that a lipophile may use the added waxy material as a facilitated channel of entry; after six days the egg may become *less* resistant to lipophilic materials. The changes in the resistance of eggs to ovicides, described below, are, of course, in themselves most important evidence supporting the conclusions in this discussion; in particular the evidence there suggests a secondary wax impregnation, rather than a wax layer.

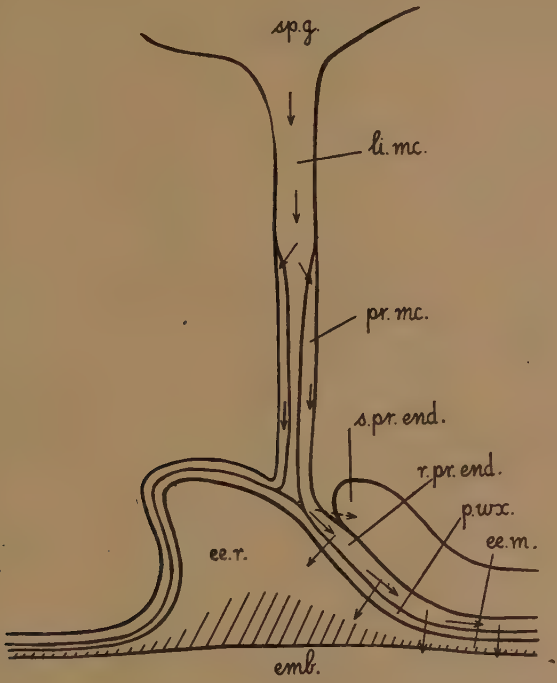

Fig. 4.—Diagrammatic section through a micropyle of an eight-day-old egg (compare fig. 3) showing the path of entry of a hydrophilic liquid. The liquid is absorbed by the protein lining of the lower micropyle (*pr.mc.*) passing to the resistant protein layer of endochorion (*rp.pr.end.*) and occasionally to the thicker soft protein layer (*s.pr.end.*). It diffuses through the primary wax layer into the epembryonic ring and membrane to reach the embryo, meeting further resistance at the secondary wax impregnation (hatched area). Other abbreviations as in fig. 3.

Summary of resistance changes.

Age (days)	Lipophiles	Hydrophiles	Cause
0–5	Increase	Increase	Epembryonic membrane.
5–7	Decrease	Increase	2° wax.
7–12	No change ...	No change ...	No change.
13–15	Decrease	Decrease	Emulsification, breakdown.

When the embryo approaches eclosion, the secondary wax is emulsified and the epembryonic material is partially broken down. These two processes appear to take place simultaneously and a return of the alcohol phenomenon indicates a decrease in the " rigidity " of the membrane meshwork, though there is no evidence for suggesting that the state of the one-day-old egg is reached. These changes would certainly make the eggs more susceptible to hydrophiles, though the removal of secondary wax may not aid the passage of lipophilic substances and this fact can only be discovered by ovicidal experiments.

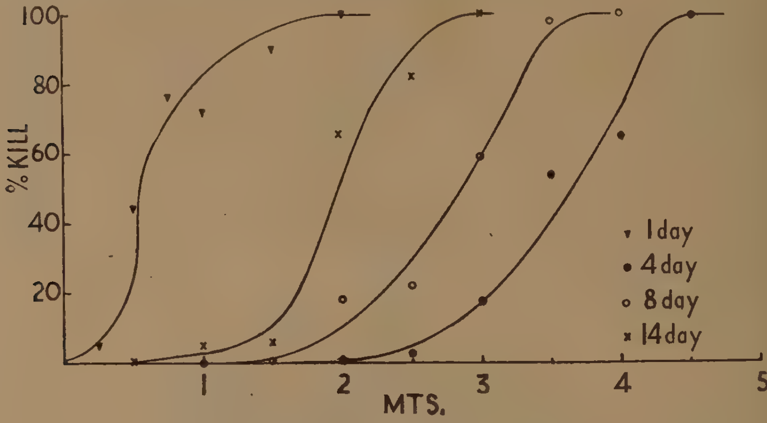

Fig. 5.—Dosage/mortality curves for *Rhodnius* eggs of four standard ages dipped in chloroform for various intervals of time.

Ovicidal Experiments.

Ovicidal experiments were carried out as described by Beament (1948); briefly, groups of 10 eggs were dipped in two c.c. of a particular liquid for a prescribed interval of time. The eggs were then dried rapidly on blotting paper, left on the bench for volatile materials to evaporate and incubated at 25°C. and 60 per cent. R.H. The eggs for each replicate were laid by females of known age and in most experiments eggs from " medium " aged females were used so that the material was as uniform as possible. As eggs were removed from cultures every 24 hours, the minimal variation between two consecutive batches could be a few minutes; the maximum could be 48 hours. A consideration of the table above shows that a batch of eggs could thus contain specimens varying very considerably from each other in the state of their internal membranes. If the cultures are disturbed more frequently than once a day, uniform oviposition is no longer obtained. For these reasons it was decided to use eggs of four standard ages. If the effects of the changes in the sub-chorial membranes are at all significant, they should be immediately obvious from a comparison of dosage/mortality curves using eggs of the following ages :—

	Primary wax layer	Fertilisation membrane	Epembryonic membrane	Secondary wax
1-day	+	+	—	—
4-day	+	+	+	—
8-day	+	+	+	+
14-day	+	+	residue	residue

Oleophiles.

Figure 5 shows dosage/mortality curves for chloroform, a volatile lipophilic liquid which is an efficient wax solvent. The increased dosage has been obtained by increasing the time of immersion. The figure shows very plainly the changes in resistance outlined above. Chloroform requires much longer to penetrate the epembryonic material; its entry is facilitated by the secondary wax impregnation and resistance again drops when the inner parts of the membrane are broken down.

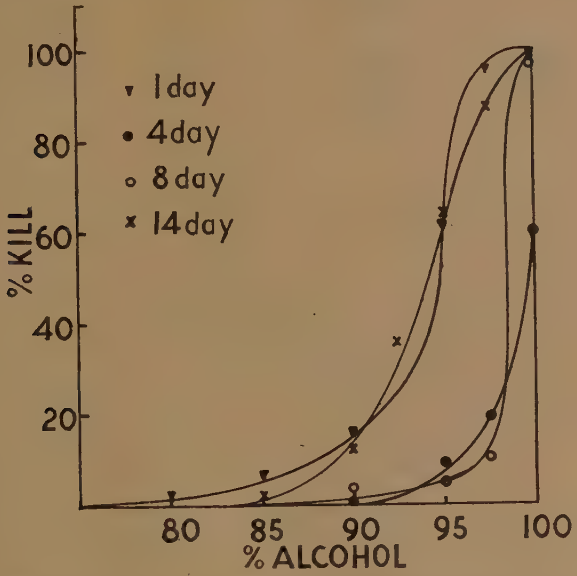

Fig. 6.—Dosage/mortality curves for eggs of four standard ages dipped in solutions of ethyl alcohol in water for periods of thirty minutes. Note steep rise in mortality when the solution becomes lipophilic and has wax solvent properties.

The distribution of the curves is important. During an investigation of the cement which covers the chorion (Beament, 1948), the difference between dosage/mortality curves for eggs having much cement, and those having little, were difference of slope. This indicated that the factor distinguishing the two groups did not effect all the members of the batch equally and some eggs of the more resistant group were killed by concentrations and exposures which were lethal to the less resistant group. In these present experiments, the differences in resistance are much more concise and must be attributed to factors which are different between age groups but constant for eggs of a particular stage of incubation. Preliminary experiments show that similar changes in resistance are obtained with benzene, xylene and other volatile wax solvents.

Text figs. 6 and 7 illustrate variations due to developmental changes in eggs treated with lipophilic liquids that possess hydrophilic properties and that are not efficient wax solvents. With these liquids, dosage can be varied in two ways; by altering the duration of dipping and by altering the concentration of the lipophile in water, keeping the immersion period constant. The toxicities of ethyl alcohol and of acetone are described. If the immersion time is varied, using absolute liquids, the changes in resistance are similar to the true lipophiles—an increase, a decrease and a further decrease. With lower concentrations in water, the liquids are no longer capable of dissolving wax, and the results then resemble the pattern of hydrophilic liquids described later.

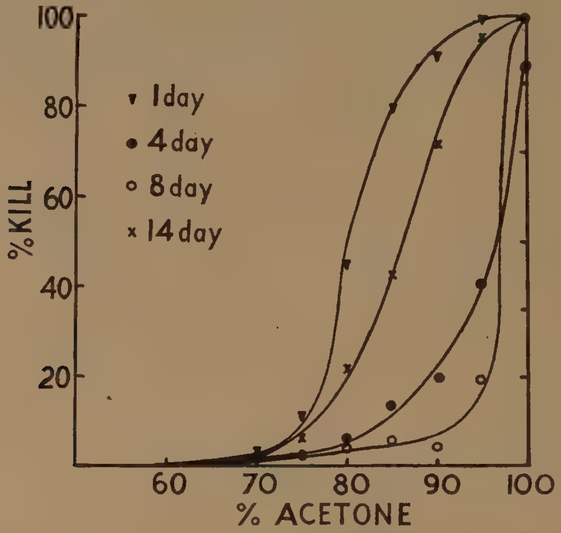

Fig. 7.—Dosage/mortality curves for eggs of four standard ages dipped in solutions of acetone in water for periods of thirty minutes. Note the steep rise in mortality when the solution becomes lipophilic.

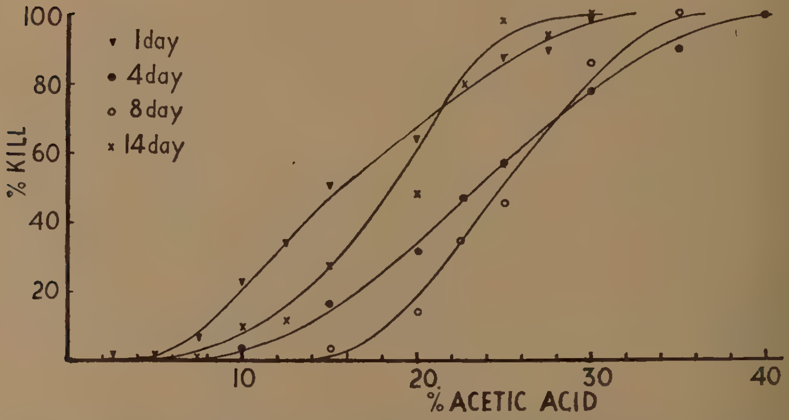

Fig. 8.—Dosage/mortality curves for eggs of four standard ages, dipped in solutions of acetic acid in water for thirty minute intervals.

Hydrophiles.

The results for aqueous solutions of acetic and formic acids (figs. 8 and 9) are typical; other experiments were performed using solutions of various salts in water and gave similar results. As the embryonic secretions are produced, the concentration of material in the applied solution must be increased if the embryo is to be killed in a fixed time. But when the membranes are impregnated with secondary wax, there is a further increase in the resistance of the egg. Fourteen-day-old eggs are much more easily killed and have almost identical properties with the one-day-old eggs. The results agree with the morphological evidence offered above.

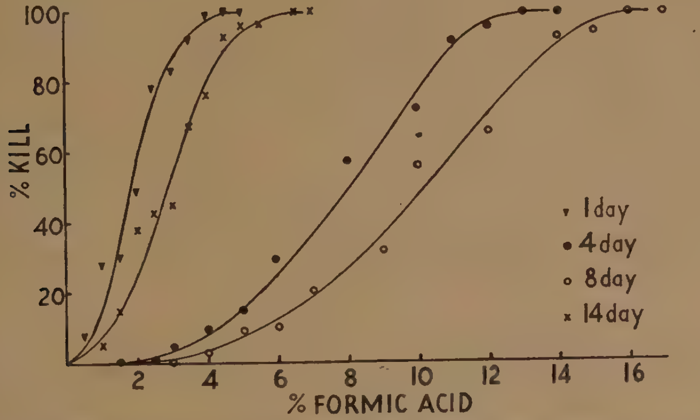

Fig. 9.—Dosage/mortality curves for eggs of four standard ages dipped in solutions of formic acid in water for periods of thirty minutes.

Wax-emulsifiers were added to certain toxic hydrophilic solutions. One-day-old eggs are more susceptible to emulsifiers *alone* than at any other stage. This evidence may be considered with hypothesis that wax repair, and the absorption of emulsifiers associated with the spermatozoa, take place through the newly-formed fertilisation membrane; the embryo's secretions are much less permeable. Predominantly oily emulsifiers enter older eggs and are probably using the impregnating wax as a path of entry.

Discussion.

Compared with many insect eggs, the egg of *Rhodnius* develops in a short period of time. In such circumstances, the changes in the protective apparatus surrounding the embryo are remarkable in their complexity. The egg is large, with a thick chorion and the danger from, for example, desiccation would be much greater in smaller eggs. But the changes do at least indicate the type of embryonic activity which may accompany resistance changes in other insect eggs—changes which are well known to applied workers (Gough, 1940; Speyer, 1934; Cory, 1929; etc.). In particular, the secretion and later emulsification of the secondary waxy material may be compared with Slifer's (1946) recent discovery that wax solvents will break the diapause in the egg of *Melanoplus differentialis*.

One further point must be considered, Beament (1948) has shown that hydrophilic materials migrate to the embryo via the layers of the endochorion. If the poisonous substance is a non-volatile solute dissolved in water, then, although some portion of the toxic material may enter the embryo, the solvent water may evaporate and immobilise some solute as a precipitate in the protein of the shell *before* the embryo has received a lethal dose. This mechanism will be more important when penetration into the embryo is slowest, e.g., after the secondary wax has been produced. Later in development, when the inner layers of the sub-chorial membrane have been broken down, this barrier no longer exists. Should the egg then absorb water, the immobilised solute in the endochorion would become a poisonous solution once more and though its concentration may be lower, it may be lethal owing to the decreased resistance of the inner membranes. Some evidence of this kind has already been obtained. Eggs were subjected to sub-lethal doses of aqueous solutions of salts of heavy metals. The eggs were eight days old with maximum resistance. They would survive this treatment if incubated under normal circumstances; but if on the fourteenth day of incubation, when most of the inner membranes have been broken down, the eggs were placed in water for about an hour, they would then fail to hatch. This result may have some bearing on ovicidal recordings in the field.

Water and solutions are absorbed through the micropylar tubes into an enclosed protein layer and the morphology of the shell is an essential part of the mechanism. Materials precipitated in the endochorion of the shell will not be leached out at all readily by immersing eggs in water. This view is, of course, applicable only to *Rhodnius* eggs and its application to eggs of other species depends on further investigation of their shell structure.

It has also been indicated (Beament, 1948 and above) that both hydrophilic and lipophilic substances may be absorbed by, and remain in, the sub-chorial layers—oils, for example, in the waxy material and hydrophiles in the protein components. When the membranes are broken down before hatching takes place, some of the absorbed materials may be released into the fluids surrounding the embryo and hence reach and kill the living material. Both this possibility and that outlined above, may account for embryos dying at times other than the period immediately subsequent to the application of the poisonous liquid. Kuenen (1946) has recorded such phenomena in eggs of *Paratetranychus pilosus* and has given other possible explanations.

Summary.

During development, membranes are added to the inner surface of the chorion in eggs of *Rhodnius prolixus*. The chemistry and permeability of the membranes have been investigated.

A true fertilisation membrane is produced immediately before oviposition when the egg is fertilised. It is attached to the inner surface of the primary wax layer, recessed into the micropylar region and covers the whole inner surface of the shell.

The membrane is very thin when first formed. It is colourless, comparatively resistant to solvents and apparently composed of " vulcanised " protein. It is semi-permeable to salt solutions but made very permeable to small molecules when immersed in absolute alcohols.

In the following five days of incubation, further material is added to the fertilisation membrane. Over the main part of the shell and cap, this does not cause a great increase in the thickness of the membrane. The added material is probably proteinaceous, with tanning and vulcanising substances and it is mostly a product of the serosa.

Opposite the inner openings of the micropyles, material is accumulated at a much greater rate and by six days incubation the membrane may be fifteen microns thick. The inner surface of the egg-shell thus becomes a uniformly ellipsoidal body. The thickened material has been called the epembryonic ring.

At about the sixth day of incubation, shortly before blastokinesis, the membrane is partially impregnated with a high-melting-point wax. This raises the " transition point " in the egg's water-loss/temperature curve from 42·5°C. to 68°C. Evidence is against this material being arranged as a second wax layer on the inner surface of the membrane.

No further changes were detected until the thirteenth day when the innermost part of the membranes are broken down by the embryo. On the day before eclosion the embryo is surrounded by a liquid containing emulsified wax and proteinaceous material. The properties of the membranes return towards those of the one-day-old egg but do not attain them; the egg hatches on the 16th day.

The changes in the membranes produce considerable changes in the apparent toxicity of ovicidal liquids; ovicidal experiments are recorded and explained. In general, the egg becomes more resistant to lipophiles over the first six days and less resistant afterwards, due to the wax impregnation. The resistance to hydrophiles increases during development due to the epembryonic layer and secondary wax, but decreases when the membranes are broken down just before eclosion.

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References.

BEAMENT, J. W. L. (1945). The cuticular lipoids of insects.—J. exp. Biol., **21**, p. 115.

——. (1946a). The waterproofing mechanism of an insect egg.—Nature, Lond., **157**, p. 370.

——. (1946b). The formation and structure of the chorion of the egg in an Hemipteron, *Rhodnius prolixus*.—Quart. J. micr. Sci., **87**, p. 393.

——. (1946c). The waterproofing process in eggs of *Rhodnius prolixus* Stähl.—Proc. roy. Soc., (B) **133**, p. 407.

——. (1947). The formation and structure of the micropylar complex in the egg-shell of *Rhodnius prolixus* Stähl.—J. exp. Biol., **23**, p. 231.

——. (1948). The penetration of insect egg-shells. I.—Penetration of the chorion of *Rhodnius prolixus*, Stå -Bull. ent. Res., **39**, p. 359.

CAMPBELL, F. L. (1929). The detection and estimation of insect chitin; and the irrelation of "chitinisation" to hardness and pigmentation of the cuticle of the American cockroach, *P. americana* L.—Ann. ent. Soc. Amer., **22**, p. 401.

CORY, E. N. (1929). Control measures for European red-mite on peach and apple.—Trans. Peninsula hort. Soc., Jan., **1929**, p. 109.

FRAENKEL-COURAT, F. & COOPER, M. (1944). The use of dyes for the detection of acid and basic groups in proteins.—J. biol. Chem., **154**, p. 339.

GODDARD, D. R. & MICHAELIS, L. (1934). A study on keratin.—J. biol. Chem., **106**, p. 605.

GOUGH, H. C. (1940). The toxicity of sulphur dioxide to the bed bug, *Cimex lectularius* L.—Ann. appl. Biol., **27**, p. 101.

HURST, H. (1943). Principles of insecticide action as a guide to drug reactivity-phase distribution relationships.—Trans. Faraday Soc., **39**, p. 390.

KUENEN, D. J. (1946). Het winterei van het fruitspint (*Metatetranychus ulmi* Koch), an zijn bestrijding.—Tijdschr. PlZiekt., **52**, p. 69.

LEES, A. D. (1947). Transpiration and the structure of the epicuticle in ticks.—J. exp. Biol., **23**, p. 379.

—— & BEAMENT, J. W. L. (1948).—Quart. J. micr. Sci., **89**, p. 291.

LISON, L. (1936). Histochimie animale.—Paris, Gauthier Villars.

LOEB, J. (1913). Artificial Parthenogenesis and Fertilisation.—Chicago.

MELLANBY, H. (1935). The early embryonic development of *Rhodnius prolixus*.—Quart. J. micr. Sci., **78**, p. 71.

——. (1936). The later embryology of *Rhodnius prolixus*.—Quart. J. micr. Sci., **79**, p. 1.

SIKES, E. K. & WIGGLESWORTH, V. B. (1931). The hatching of insects from the eggs and the appearance of air in the tracheal system.—Quart. J. micr. Sci., **74**, p. 165.

SLIFER, E. H. (1937). The origin and fate of the membranes surrounding the grasshopper egg.—Quart. J. micr. Sci., **79**, p. 493.

——. (1946). The effect of xylol and other solvents on diapause in the grasshopper egg.—J. exp. Zool., **102**, p. 333.

SPEYER, W. (1934). Obstbaumkarbolineum als Schädlingsbekämpfungsmittel.—Z. angew. Ent., **20**, p. 565.

STONE, P. C. & LAHUE, D. W. (1946). Rapid vacuum fumigation of the body louse with methyl bromide.—J. econ. Ent., **39**, p. 581.

WEBB, J. E. & GREEN, R. A. (1945). On the penetration of insecticides through the insect cuticle.—J. exp. Biol., **22**, p. 8.

WIGGLESWORTH, V. B. (1945). Transpiration through the cuticle of insects.—J. exp. Biol., **21**, p. 97.

——. (1947). The epicuticle of an insect, *Rhodnius prolixus* (Hemiptera).—Proc. roy. Soc., (B) **134**, p. 162.

ACRODENDROPHILIC MOSQUITOS OF THE LANGATA FOREST, KENYA.

By P. C. C. Garnham.

Y.G.

Reader in Medical Parasitology, University of London.
(Late of the Division of Insect-Borne Diseases, Nairobi.)

Langata Forest is a small, detached forest, 11 square miles in area, of the African plateau type, lying 5 miles west of Nairobi at an altitude of 6,000 ft. The forest is mostly secondary and although of evergreen character, much foliage is lost during the long dry seasons. The indigenous trees include olives (*Olea chrysophylla*), crotons (*Croton megalocarpon*), muhugu (*Brachylaena hutchinsonii*) and cape chestnut (*Calodendron capense*).

The forest is much broken by plantations of exotic trees and by plots occupied by European settlers. The rainfall is usually rather low; during 1946 the figures in inches were as follows: January 0·1, February 0·4, March 0·7, April 6·0, May 3·3, June 0·4, July 0·2, August 1·6, September 2·4, October 6·0, November 3·1, December 2·5. For a large part of the year, the climate is cool and the nights are cold, the temperature dropping to 12°C.

A human case of yellow fever was traced to Langata in 1943. Specimens of human and monkey sera from the area were sent to the Yellow Fever Research Institute, Entebbe, Uganda, and three human and four (*Colobus*) monkey samples were found by the Institute to contain protective bodies against the virus. These results indicated that a mild focus of yellow fever existed in the forest, which is probably the highest place in Africa for the disease; further east, at this altitude, Liégeois, Rousseau and Courtois (1948) noted that yellow fever was absent.

Observations on the forest mosquitos were begun in 1944 and the incidence of larvae in tree-holes and in artificial containers and of adults caught in the bush was measured. *Aëdes aegypti*, L., were found in both larval and adult forms; a few new species were discovered and recorded by Mrs. E. van Someren (1946), but otherwise the information was not particularly interesting. In 1946, the observations were therefore extended in order to determine the incidence of adult mosquitos in the upper strata of the forest.

A platform was built in the upper branches of a tree 30 ft. from the ground and a ladder was nailed to the trunk to provide easy access. The tree was in secondary forest near the Forest Station and was growing just beside a wide track. The canopy was much broken and the foliage was sufficiently luxurious to shade the platform only during the wet seasons; at other times of the year it provided partial shade.

This platform was used for the collection of mosquitos alighting on an African child from 5-8 p.m. in the evening and from 7-10 a.m. in the morning. Six such catches were performed every week from May, 1946, until March, 1947. The periods occupied the twilight hours or just before or after. At other times of the day, mosquitos were rarely found to attack man.

The results of the platform catches are shown in Table I. Catches on platforms on another tree in the following year revealed a similar distribution of species, with *Aëdes deboeri*, Edw., again heading the list (171 *deboeri* in the evening and 69 in the morning catches).

Ground catches during the same period revealed a wider range of mosquitos, including many species of *Culex*, but the relative density of individual species was much altered. Nearly 2,000 *A. dentatus* were captured but only nine *A. deboeri*, five *A. ingrami* and no *A. pulchrithorax*.

Seasonal fluctuations in numbers occurred; *A. deboeri* in the platform collections being most numerous immediately after the long rains. The catches at any season were nearly always more prolific in the evening than the morning.

TABLE I.

Female *Aëdes* caught on 30-foot Platform in Langata Forest, Kenya, May 1946–March 1947.

Species	Number caught in morning	Number caught in evening
Aëdes (*Finlaya*) *ingrami*, Edw. ...	17	17
Aëdes (*F.*) *pulchrithorax*, Edw. ...	8	19
Aëdes (*Stegomyia*) *keniensis*, van S. ...	0	1
Aëdes (*S.*) *deboeri*, Edw.	28	154
Aëdes (*S.*) *aegypti*, L.	1	0
Aëdes (*Aëdimorphus*) *marshalli*, Theo. ...	0	1
Aëdes (*A.*) *haworthi*, Edw.	0	1
Aëdes (*A.*) *dentatus*, Theo. (and group)	6	22

Discussion.

The results of this investigation are on too small a scale to allow definite conclusions to be drawn, but two facts emerge which are of some interest. Firstly *A. aegypti* is present in the forest and secondly *A. deboeri* is a relatively common mosquito in the upper strata of the forest, though not at ground level. In another forest (Kaimosi), of about the same altitude, it was shown (Garnham, Harper & Highton, 1946) that *A. africanus*, Theo., a known vector of yellow fever in nature (Haddow & others, 1948) was the principal acrodendrophilic species. *A. africanus* is absent in Langata, though the tree (*Craibia elliottii*) in which it characteristically breeds is common. Bailey (1947) has suggested that *A.* (*Diceromyia*) *adersi*, Edw., may be yellow fever vector in the coastal forests of Kenya; this species also is not found in Langata. In the absence of further evidence, it is impossible to state more than that *A. deboeri* may be the jungle vector of the disease in Langata.

References.

Bailey, K. P. (1947). A preliminary note on the sylvan mosquitos of Gede.—E. Afr. med. J., **24**, pp. 38-41.

Garnham, P. C. C., Harper, J. O. & Highton, R. B. (1946). The mosquitos of the Kaimosi Forest, Kenya Colony, with special reference to yellow fever.—Bull. ent. Res., **36**, pp. 473-496.

Haddow, A. J., Smithburn, K. C., Dick, G. W. A., Kitchen, S. F. & Lumsden, W. H. R. (1948). Implication of the mosquito *Aëdes* (*Stegomyia*) *africanus*, Theobald in the forest cycle of yellow fever in Uganda.—Ann. trop. Med. Parasit., **42**, pp. 218-223.

Liégeois, P., Rousseau, E. & Courtois, C. (1948). Complément d'enquête sur la distribution de l'immunité antiamaril naturelle chez les Indigènes du Congo Belge.—Ann. Soc. belge Med. trop., **28**, pp. 247-267.

van Someren, E. C. C. (1946). Ethiopian Culicidae—descriptions of the adults and pupae of two new species and two sub-species of the genus *Aëdes* from East Africa (Diptera).—Proc. R. ent. Soc. London, (B) **15**, pp. 1-6, 2 figs.

THE USE OF TRAPS AGAINST TSETSE IN WEST AFRICA.

By K. R. S. Morris, D.Sc.
Medical Entomologist, Gold Coast,
and
M. G. Morris, B.Sc., Ph.D.
Research Assistant, Trypanosomiasis Campaign, Gold Coast.

(Plates XXIII & XXIV.)

Contents.

	PAGE
Introduction	491
Locality	493
The Experiments	494
The Animal Trap	495
Modifications	497
Comparison with other Traps	500
The Importance of Siting	501
Factors for Successful Siting	502
Comparison of Traps with Flyboys	506
Trapping for the Control of Sleeping Sickness	516
Summary	525
Acknowledgements	527
References	527

Introduction.

In the first, and still almost unique, completely successful campaign for the eradication of tsetse-flies, trapping was one of the means employed for destroying the adult flies. This took place in the Island of Principe, and was begun in 1906 when Maldonado, a plantation owner, observing the frequency with which tsetse settled on the backs of labourers cutting grass, ordered them to wear on their backs black cloths smeared with bird-lime. By this simple technique 133,800 *Glossina palpalis*, R.-D., were trapped in one plantation between April 1906 and the end of 1907. The method was adopted by the Portuguese Sleeping Sickness Commission and men were sent in twos to any estate where the high incidence of flies was making outdoor activity intolerable by day. The reduction of the daily catch from 1,500-2,000 flies to 15-20 by the end of a week gives an idea of the severity of the infestation in some localities and the success of the control measure (Maldonado, 1910). The process was continued, together with other methods of control, on a wider scale by da Costa, in a determined effort to exterminate the fly from the island and by 1914 not a fly remained (da Costa & others, 1916).

The main work on trapping has been carried out against the game tsetse in East Africa. Harris designed a trap based on the tendency of the fly, when attacking an animal, to dive to the lower parts of its belly. He attributed this to a response to the effect of light and shade and emphasised that this factor must be remembered when trapping sites are selected (Harris, 1930). At Umfolosi, in Zululand, he attacked *G. pallidipes*, Aust., by the use of traps on a vast scale and so reduced the population that, whereas in 1931 over 7 million flies were caught by 487 traps, in 1937 only 57 thousand were caught in a total of 8,928 traps. By 1938 the reduction was apparently still continuing but immigration had so far prevented eradication from being complete (Harris, 1938). Jack (1939), working with *G. morsitans*, Westw., in Southern Rhodesia, made several trials in 1930 and 1931 with both Harris traps and traps of his own design built specially to attract the flies into a dark interior. Swynnerton was already experimenting with traps in 1930, basing his original models on the simple catching screens that he had been using since 1922. His team of workers devised and tested a large variety of patterns, and from this work Swynnerton drew a number of conclusions on the factors necessary for successful trapping and the possibilities of controlling various species. His Single Screen trap was more effective than the Harris trap for *G. palpalis* and *G. pallidipes*, and a steady decrease in the numbers of the latter in a block of fly-belt near Shinyanga was brought about in three months by the use of 100 traps (Swynnerton, 1936, pp. 244-260). C. W. Chorley (1933) specialised in various modifications of the Crinoline trap which have the merits of simplicity and cheapness of construction as well as lightness and portability. A thorough test of the use of trapping for the eradication of *G. palpalis* was made by Symes (1935) in Kenya. He found that traps were of most use in places where the fly density was high, but that hand-catching had to be substituted, and was more economical, when the numbers were reduced. T. W. Chorley, working in Uganda, has recently devised a simple screen smeared with grease which, when carried behind a bicycle, is effective in taking either *G. pallidipes* or *G. morsitans* according to the height above the ground. He also used a " Fisherman's Hat " trap for taking *G. palpalis* (personal communication). Nowhere in East Africa has any great success been recorded in the control of *G. morsitans* by traps.

Since da Costa's work few serious attempts have been made to trap the West African species of *Glossina*. In 1931 one of the writers tried Harris traps against *G. longipalpis*, Wied., at Takoradi in the Gold Coast, but did not find them very effective in the dense secondary bush of that locality. In open glades, traps would catch the flies in their immediate vicinity in the course of a few days, then no more were caught until the traps were moved to new sites although flies were abundant in the surrounding bush. In 1933-34 Henrard carried out experiments on an island in Stanley Pool on the Congo using various types of trap, based on the Harris model, against *G. palpalis*. He found that the traps caught much more successfully if they were situated in places frequented by people or animals and he therefore concluded that, although traps could not by themselves constitute a method of eradicating the flies, they might be used with advantage at such places as river crossings, water-holes, etc. (Henrard, 1934). This work was taken further by Lewillon in the Kwango district of the Belgian Congo during 1941-42, with various traps of his own design, the most successful being the " Mannequin ". He concluded that the systematic trapping of *G. palpalis* would be a reasonable method of controlling sleeping sickness and recommended this procedure wherever the nature of the country permits (Lewillon, 1945). From 1934 to 1936 Dr. A. W. Macpherson developed several forms of trap coated with bird-lime which he tried out in the forest conditions of Ashanti. Zumpt advocated trapping as a promising means of controlling *G. palpalis* in plantations in the Cameroon Mountains but did no trapping experiments himself (Zumpt, 1937).

The work described in this paper is on the use of traps against *G. palpalis* and *G. tachinoides*, Westw., the principal vector of sleeping sickness in West Africa, and it was carried out in Lawra district in the north-west corner of the Gold Coast; it was begun in 1940 and some additional experiments were still in progress at the time of writing. Swynnerton's A.S.B. trap, Chorley's Crinoline trap and the Harris trap were used. At the same time, taking into consideration the difference between the feeding habits of *G. palpalis* and *G. tachinoides* and those of the East African game tsetse, a new type of small Animal trap was devised.

The present paper describes the Animal trap, compares its efficiency under local conditions with that of the other traps, and discusses its possibilities as a factor in the control of *Glossina*.

Locality.

Lawra district, where the experiments were carried out, lies in the Savanna Forest zone in the north of the Gold Coast. The climatic conditions show a marked contrast between wet and dry seasons, since the annual rainfall, which varies between 40 and 50 inches, is restricted almost entirely to the period April-October. Maximum temperatures average 85°F. during the rains and rise to a monthly mean of 95°F.–100°F. at the beginning and end of the dry season. The dry season is broken by two or three months of cool, hazy weather, usually from December to February, when the dry harmattan wind blows intermittently from the north-east causing extremely low humidities.

Intensive cultivation and grazing gives the countryside an open, steppe-like appearance with abrupt transitions between the dense evergreen vegetation of the watercourses and the adjacent open farmland, grassland or poor savanna woodland.

The evergreen vegetation fringing the rivers and large streams forms the fly-belt or permanent habitat of *G. palpalis* and *G. tachinoides*. During the dry season the flies rarely venture more than about a hundred yards from this cover. During the rains, however, they extend their range considerably, travelling many miles along more open watercourses and colonising temporary habitats in clumps of evergreen or deciduous trees. On large rivers such as the Black Volta and the lower reaches of its major tributaries, which hold abundant water throughout the year, the fringing vegetation forming permanent fly-belt is more or less continuous for many miles along the banks. In width it is never more than 50-60 yards and in many places as narrow as 5-10 yards. Within this type of habitat it is easy to recognise certain regular feeding grounds where hungry flies concentrate, but one is liable to be bitten wherever one enters fly-belt. The feeding grounds are usually openings in the river-bank vegetation varying in length from a few yards to a few hundred yards. These openings are nearly always artificial, and are found where a settlement is, or has been, situated close to the river, or where people and animals go down to the water-side regularly for drinking, drawing water, washing, fishing, or to cross the river. Feeding grounds also occur where a well-used road or path skirts the fly-belt in following the river bank.

Higher up the tributaries the stretches of permanent fly-belt tend to become more restricted, sometimes to a mile or so in length, sometimes to patches of a few hundred yards. The vegetation which forms the fly-cover also becomes lower and narrower, though retaining the dense evergreen impenetrable character of the Volta fly-belt. These stretches of permanent habitat are frequently, but by no means always, associated with large pools which retain water all through the dry season. Since pools always form a focus of activity for the local population they afford regular and attractive feeding grounds for the tsetse, and the activity of the fly communities therefore tends to concentrate at the established water-holes and road crossings.

On the upper reaches of the rivers and along the smaller streams a third type of permanent fly-belt occurs, even more limited in extent than the second. The thick vegetation is restricted to groves which invariably enclose permanent water-holes and are often close to villages. They are usually about an acre in extent and are isolated from other fly-belts by several miles of perfectly open river. In vegetation these groves resemble miniature patches of forest with tall, evergreen shade trees, such as mahogany, *Ficus* spp., *Syzygium guineense* and *Celtis integrifolia,* and low underbrush. The water-holes in groves are usually numerous small excavations, and they are visited all day and every day by natives and by miscellaneous cattle, sheep and other animals which come to take advantage of the shade and water in the heat of the day. In addition, many of the groves are sacred and one often sees a few apparently harmless crocodiles, feared by neither man nor beast. Conditions are thus very favourable to the tsetse, and they can be found feeding round the periphery of the grove and inside it, wherever open spaces afford good visibility of their hosts.

In this brief account of the habitats of *G. palpalis* and *G. tachinoides*, the feeding grounds have been described in detail since it is only there that trapping can be effective. Furthermore, it is in such places of intimate fly-man contact that the transmission of trypanosomiasis is most likely to occur and, therefore, that the necessity for protection from the tsetse is greatest.

The Experiments.

The primary object of the investigation was to find a means of controlling *G. palpalis* and *G. tachinoides* in sacred groves, which constitute a special problem in many parts of the Northern Territories. These groves are pernicious foci for the dissemination of trypanosomiasis since they are centres of concentration of both villagers and tsetse. Rigorous taboo opposes the cutting of any vegetation within them, and one or two early attempts to introduce clearing methods resulted in the loss of confidence and co-operation of the people.

As these patches of fly-belt are well isolated by open country and are very limited in extent, it seemed possible that direct attack on the adults might eliminate the flies. It was found that teams of fly-boys, catching every day, could rapidly reduce the tsetse populations and, in 8–12 weeks, bring them almost to the point of elimination, but always an odd fly or two appeared sooner or later, ready to form the nucleus of a new population whenever vigilance was relaxed. Trapping would be considerably cheaper than catching by fly-boys and if it proved as effective it might provide a system of control with possibilities of extension. Four groves situated on a small stream near Lawra, were chosen as trapping sites, and series of traps were kept under observation for four years. Different types of traps were tested over long periods and at times changes were made in the numbers of traps used to see whether concentrated effort could achieve final eradication. Routine monthly catches by a team of fly-boys provided additional useful data.

Trapping round water-holes and river crossings might also have a protective value by reducing the man-fly contact, and might perhaps prove a useful adjunct to short protective clearings by intercepting a proportion of the flies that inevitably enter from the adjacent fly-belt. Series of trials were, therefore, conducted at a fishing camp and two canoe crossings on the Black Volta, and on the Kamba at two well-frequented road crossings. Because of the much higher numbers of tsetse present, the sites were more valuable than the groves in supplying conclusive evidence of the comparative values of different shapes, sizes and colours of traps and in enabling a decision to be made as to the optimum design for the Animal trap.

The selection of the type of trap to be used was governed by a consideration of the customary food hosts of *G. palpalis* and *G. tachinoides* in populous country. In such regions *G. palpalis* feeds almost exclusively on man and domestic animals, *G. tachinoides* feeds on the same hosts and, in addition, on reptiles. Thus Chorley's Crinoline trap might be mistaken by a tsetse for the body of a man; small forms of Harris or Swynnerton's Screen traps might represent the local dwarf breeds of cattle, but both were too big to resemble what are by far the most numerous of the food hosts along the banks of rivers, sheep, goats and, in the more thinly populated parts, duikers. A new form, the Animal trap, was devised and was built to represent a small animal the size of a goat. This was tried out against Chorley's Crinoline traps made 18 inches in diameter and 24 inches long in khaki and black material, Harris' traps built to two-thirds standard size, and Swynnerton's A.S.B. traps with screens 4 ft. and 2 ft. long by 2 ft. deep.

For the purpose of making comparisons between the different traps several of each were kept in operation simultaneously at each trapping locality. Positions were chosen to give, as far as could be judged, equal opportunities in each case. But in practice positions vary considerably in productivity, so the traps were interchanged periodically in order to level out the advantages of position. The traps were visited twice a week by regular trap-boys, who removed, identified and recorded the flies. Minor repairs were carried out on the spot and any trap that was out of action was replaced on the next visit.

In the estimates of the relative values of the traps, the catches for all traps of any one kind were averaged and standardised to 30 trap-days per month. The comparison of any two types was based on data from traps working simultaneously in the same locality. The standardised catches for the periods of the trials were then summed for all localities. Unless otherwise stated, this is the method adopted in the compiling of the results detailed below.

The Animal Trap.

Construction and Maintenance.

The essential feature of the Animal trap is the cylindrical shape of the body which, with the resulting high-lights and shadows on the upper and lower surfaces, gives the trap the appearance of an animal's trunk and makes it a conspicuous object when viewed from any angle.

After trials with various sizes the trap was eventually reduced to a standard size and pattern of functional simplicity, combining maximum catching power with strength and with ease in construction and handling. The first traps were made with whole skins of animals complete with heads, tails and legs, rather like miniature Trojan horses, but trials with simpler forms showed that this degree of naturalism was appreciated more by the entomologist than by the tsetse.

The design of the trap in its standardised form and dimensions can be better understood from the diagram (fig. 1) and photographs (Pl. XXIII, figs. 1 & 2) than from a written description. Some practical details of its construction, however, may be useful. The vertical ends of the rectangular body-frame are made

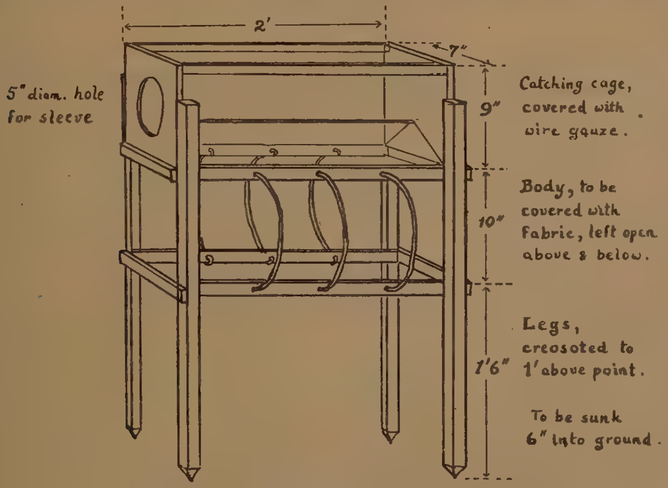

2'
7"
5" diam. hole for sleeve
9"
Catching cage, covered with wire gauze.
10"
Body, to be covered with fabric, left open above & below.
1'6"
Legs, creosoted to 1' above point.
To be sunk 6" into ground.

Fig. 1.—Construction and dimensions of the Animal trap.

from 3-ft. lengths of 1 in. × 1 in. termite-resistant wood, arranged so as to project 1 ft. 6 ins. downwards to form the legs of the trap, and 6 ins. upwards to hold the wire-gauze catching cage firmly in position without the need for its being nailed to the body. This allows easy removal and replacement of the cage for repairs. The cage is of the usual non-return pattern, with a 5-in. diameter hole at one end, closed with a sleeve, to allow access for the removal of flies and adjustments to the entrance slit. The sides and ends of the body frame are covered with hessian or other cloth, the bottom and top being left open for the entry of the flies and their passage into the cage. Three ribs of stout galvanised wire on each side are sufficient to give the body its essential rotundity.

It was found that treating the woodwork with creosote had a repellent effect on tsetses which took a considerable time to wear off, so only the lower extremities of the legs are treated. Sinking the legs six inches into the ground gives the trap sufficient stability to prevent its being washed away by sudden floods, butted over by neighbourly goats, or otherwise upset. At the same time this brings the body of the trap about one foot above the ground, roughly the height of a standing or grazing goat; this was the position found to be most favourable for attracting *G. palpalis* and *G. tachinoides*.

In contrast to the experiences of Swynnerton and others in East Africa, suspended traps, which are given some movement by the wind, were no more effective than fixed traps. This may be explained by the fact that tsetses of the *palpalis* group, unlike those of the *morsitans* group, come more readily to a stationary than to a moving host. The rigid method of fixing, moreover, gives the practical advantages of a self-contained trap, easily set in place and as easily transferred to a new position. Suspension necessitates ropes which rot and fray, and supports which, because open feeding grounds are so often the best catching places, may have to be arranged artificially.

A particular advantage of the Animal trap is that it combines lightness and portability with strength. A trap-boy can carry three on a bicycle or if a large number are needed they can be closely packed in a kit-car without fear of damage. Again, as the traps are often situated close to the water's edge they are liable to be washed away by sudden floods in the rainy season, but as they float they can usually be rescued down the river, more or less intact, when the water subsides.

The trap is simple to construct and maintenance is very light. The most important point is constant attention to the entrance slit of the catching cage, which must be, throughout its length, just wide enough to admit the flies but not wide enough to let them escape. This can be ensured by links of fine wire fixed at intervals along the slit, holding it open but preventing too wide a gap. Observation has shown that if the entrance slit is too wide quite a number of the tsetse, which are very active in the cage for an hour or so, find their way out and escape. For this reason it is essential that the inside of the cage should be easily accessible and that trap-boys should be impressed with the importance of always keeping the slit adjusted to the right width. The grass and herbage below a trap and for a few yards round it must be cut short so as to maintain its visibility and a sharply contrasting shadow on the ground. The cleared space is also important as a protection from burning in the dry season when grass fires are frequent. Unlike the game tsetse in East Africa, *G. palpalis* and *G. tachinoides* are never caught in such numbers as to necessitate their frequent removal from the traps. In any case, the flies die in a few hours when the cages are in direct sunlight so that, if traps are being used for control and not experimental purposes, visits once a week or even once a fortnight are sufficient for maintenance. Trap-boys always carry hammer and nails, needle and thread and spare cloth on their rounds and effect minor repairs on the spot. Complications have occasionally arisen through snakes or harmless but equally feared geckoes getting into the cage. In practice damage was infrequent and very seldom was it done wilfully. The sleeves were sometimes removed, very rarely the cloth, though this was occasionally slashed or punctured by little boys who like the traps as targets for their arrows. Our record was a duiker-skin trap, successfully stalked and shot by an enthusiastic hunter.

Finally, as well as being easy to make, the Animal trap is relatively inexpensive. In 1946 the cost, including materials, which had to be transported 350 miles from Kumasi, and labour was 9s. 6d. per trap. At the same rates for materials and labour the costs of the other traps would be: Harris trap, 19s. each; the smaller size of Swynnerton's A.S.B. trap, 14s. 6d.; Chorley's Crinoline trap, 5s.; although at the time the models of these traps were made for experimental purposes the actual costs were considerably less. The most expensive item in the first three was the wire gauze for the trapping cage, 6s. 8d. in each case, which thus represents over two-thirds of the cost of the Animal trap, less than half the cost of the A.S.B. trap and one-third the cost of the Harris trap.

Modifications.

(i) *Structure.*

In the course of early observations on the reaction of tsetse to the Animal trap it was noticed that some of the flies, after wandering over the body of the trap, make their way upwards and eventually fly off, instead of walking under the belly and so getting inside. To overcome this a modification in structure was tried. An awning of wire gauze was made, to project outwards and slightly downwards from the bottom of the cage, and a space of about an inch was left between the top of the body and the cage, giving ingress to the latter from the outside of the body. The principle was similar to Jackson's modification of the Harris trap into the J.V. and S.V. traps (Swynnerton, 1933). This modified trap, however, proved less successful than the simple traps, probably because the awning spoiled the effect of contrasting light and shadow as well as somewhat obscuring its visibility (Pl. XXIII, fig. 4). The relative effectiveness of the two forms is shown by a comparison of their catches standardised according to the method stated above :—

Aggregate of 23 trap-months in 5 localities.

	palpalis	*tachinoides*
Animal Trap with awning	45	362
Animal Trap without awning	259	1,327

To this marked inferiority in performance was added increased complexity and expense in construction, so the modification was abandoned.

(ii) *Size.*

After a series of trials with traps of various sizes the standard size of a 2 ft. long body raised 1 ft. from the ground was adopted as being most suitable both for efficiency and for convenience in handling. This size was originally chosen because it could be covered with the skin of a local small-sized sheep or goat. Much later during the course of the investigation it was found that the Animal traps were catching a smaller proportion of *G. palpalis* than of *G. tachinoides* compared with the frequencies of the two species in the flyboys' catches. It was thought that this might be due to the difference in the feeding habits of the two species, *G. palpalis* preferring man and the domestic animals associated with man, *G. tachinoides* preferring smaller mammals and reptiles. Some Animal traps of double the usual size were built, 4 ft. long and 3 ft. high at the shoulder, which is roughly the size of the local dwarf cattle. Great care was taken in this trial to ensure that the result was not influenced by inequalities of the trap sites. The inferiority of the large trap is quite evident :—

Aggregate of 16 trap-months in 3 localities.

	palpalis	*tachinoides*
Standard Animal Trap	152	2,296
Double-sized Animal Trap	113	1,595

(iii) *Colour and Material.*

The choice of materials for covering the body of the trap was limited by war-time restrictions in supply, but extensive tests were made with both rough- and smooth-textured fabrics in dark and light colours.

Since the trap was designed to attract tsetse by its resemblance to the fly's natural hosts, the first models were covered with the skins of sheep, goats and small antelopes. As little difference was found in the catching powers of the traps with these different coverings, goat skins, being the cheapest and the most readily obtainable, were used exclusively in the later trials. Animal skins, however, had several disadvantages : they were relatively expensive, they often could not be obtained in sufficient numbers, and they rotted during the rains. If trapping were to be a practical proposition, and especially if it were to be undertaken in the damp conditions of forest country, it was essential to find a substitute covering more durable than skins. Cloth with a hairy or woolly surface, such as blanket or rough flannel, was known to be superior to smooth cloth in attracting tsetse to screens (Swynnerton, 1933), but, because of difficulties of supply, cheaper and more readily available materials had to be sought. Black and khaki drill and black and natural hessian were used to test the values of smooth and rough materials and dark and light colours. Little other choice was possible at the time. Surprisingly enough, all these, when tried out against animal skins, proved superior in catching power. The most extended trial was between traps covered with goatskins and those made with black drill :—

Aggregate of 22 trap-months in 3 localities.

	palpalis	*tachinoides*
Traps covered with goatskin	14	761
Traps covered with black drill	23	1,007

The effect of difference in colour was less than had been anticipated and, in the case of the standard small Animal traps, was apparent with smooth fabric only :—

Aggregate of 29 trap-months in 4 localities.

	palpalis	*tachinoides*	*morsitans*
Traps with khaki drill	7	3,076	424
Traps with black drill	8	2,737	454

With rough surfaced coverings no difference arising from colour was found with standard traps, but the tests with the large 4 ft. long Animal trap gave an interesting result with a marked superiority of black over light brown :—

Aggregate of 16 trap-months in 3 localities.

	palpalis	*tachinoides*
Small animal trap, natural hessian ...	161	2,278
Small animal trap, black hessian ...	142	2,314
Large animal trap, natural hessian ...	93	1,433
Large animal trap, black hessian ...	133	1,756

The only explanation that can be offered is that the tsetse is accustomed (conditioned?) to goats and sheep of a considerable range in colour whereas the cattle on which it feeds are predominantly black or black and white. The large black trap could be taken for a cow more readily than could the natural hessian trap which resembles more nearly an antelope. It would be interesting to observe the reaction of *G. tachinoides* in an exactly similar experiment carried out in a locality where the fly would have no contact at all with man and his domestic animals.

Rough fabric proved to be decidedly better than smooth. For example, natural hessian attracted 20% more *G. tachinoides* than khaki drill :—

Aggregate of 22 trap-months in 3 localities.

	tachinoides	*morsitans*
Traps covered with khaki drill ...	1,520	362
Traps covered with natural hessian ...	1,809	392

Tested against black drill, hessian was found to be twice as effective :—

Aggregate of 35 trap-months in 3 localities.

	tachinoides	*morsitans*
Traps covered with black drill	1,028	367
Traps covered with natural hessian ...	2,054	537

It is in the nature of what one would expect that natural hessian should be more successful than black drill with *G. morsitans*. That the difference is not more striking may be due to the fact that the traps used in this experiment were the small Animal traps whereas *G. morsitans* feeds on the larger antelopes. When the investigation was begun *G. morsitans* was almost completely unknown in the district, but in 1941 it appeared in some localities, from the western side of the Black Volta, and was soon multiplying rapidly and spreading through unpopulated areas. By the beginning of 1946 a policy of harassing the large game animals and not allowing them to remain in any one place brought about a quick reduction of the *G. morsitans* population and its virtual elimination. When the double-sized trap was tested against the standard trap it was no longer possible to observe the reaction of *G. morsitans*.

All these comparisons of colour and texture relate to experiments made in very open clearings on the banks of the Black Volta and Kamba rivers where, with direct sunlight for most of the day, lighting and visibility of the traps were both excellent. In the case of the groves, many of the traps were stationed within the grove and, unavoidably, the lighting conditions were much poorer. The catches made by standard animal traps in four groves may be summarised as follows :—

	palpalis	*tachinoides*
Aggregate of 72 trap-months :		
Traps covered with khaki drill ...	147	262
Traps covered with black drill ...	121	219
Aggregate of 62 trap-months :		
Traps covered with khaki drill ...	92	226
Traps covered with natural hessian ...	102	206
Aggregate of 72 trap-months :		
Traps covered with black drill ...	121	239
Traps covered with natural hessian ...	142	249

These results indicate that with traps operating in a poor light, e.g., under canopy, while colour still plays some part in determining their attractiveness, the texture of the material is less important.

Comparison with other Traps.

Accurate comparisons of the catching abilities of the four different types of traps were made during the first two years of experiment on the Black Volta and Kamba rivers, where *G. tachinoides* was abundant but *G. palpalis* less so. The majority of traps used in attempts to reduce tsetse populations in sacred groves were Animal traps. A few Crinoline and A.S.B. traps were in use at the same time and although these were not worked on a routine of regularly changed positions they were always placed in what were judged to be good catching sites. As the observations were continued for more than three years it is considered that the results are of value as a basis for comparisons.

Harris traps proved to be of very little use for catching *G. tachinoides* under the local conditions, and valueless against *G. palpalis* :—

Aggregate of 19 trap-months at 2 Volta-side localities.

	palpalis	*tachinoides*
Animal traps	124	1,170
Harris traps	1	50

It would have been interesting to measure the relative merits of the two traps in respect of *G. morsitans*, but by the time this fly appeared in the district the use of Harris traps had been discontinued.

Swynnerton's A.S.B. trap proved better than the Harris trap but it, too, was inferior to the Animal trap, and caught only one-ninth as many flies during prolonged trials :—

Aggregate of 76 trap-months in 6 riverside localities.

	palpalis	*tachinoides*
Animal traps	408	4,046
Swynnerton's A.S.B. traps	19	470

Comparatively speaking, the A.S.B. traps did very much better in the groves where the Animal traps caught only 2½ times as many flies as the former :—

Aggregate of 149 trap-months in 4 groves.

	palpalis	*tachinoides*
Animal traps	320	717
Swynnerton's A.S.B. traps	138	295

The poorer lighting and visibility in the groves may well account for this difference, as the rotundity and the shadow effects of the Animal trap would be much less apparent under shade, and the flat screen of the A.S.B. trap would then be nearly as effective an attractant.

In open clearings Crinoline traps were slightly better than A.S.B. traps for both species of tsetse :—

Aggregate of 47 trap-months in 4 riverside localities.

	palpalis	*tachinoides*
Animal traps	348	3,021
Chorley's Crinoline traps	22	483

Under the shady conditions of the groves it appeared that the effectiveness of Crinoline traps was heightened in the case of *G. palpalis* but reduced for *G. tachinoides* :—

Aggregate of 33 trap-months in 4 groves.

	palpalis	*tachinoides*
Animal traps	60	160
Chorley's Crinoline traps	13	20

It might be that in a dim light *G. palpalis* would mistake a vertical black cylinder for a native torso, but as the total numbers of flies involved are so small this difference may have little significance.

Later in the course of the experiments, when it was found that *G. palpalis* was less attracted to Animal traps than might have been expected from the numbers shown to be present by fly-boys, trials were made with " Man traps " like enlarged Crinoline traps of a size to represent a human body with and without legs, but these proved inferior to Animal traps for both *G. palpalis* and *G. tachinoides*.

With both *G. palpalis* and *G. tachinoides* the ratio of males to females among the flies caught was not influenced by the type of trap.

The Importance of Siting.

For successful trapping correct siting is of paramount importance. This was emphasised by Swynnerton (1933). So important is the question of siting that an inferior type of trap placed in a good position may consistently catch more flies than a trap with superior catching power but in a poor site. So exacting is the question of siting that identical traps can be placed in what appear to be equally good positions in the same feeding ground, perhaps only a dozen yards apart, yet one will make catches four or five times as high as the other. For the recognition of good trapping sites it is essential to have comprehensive knowledge of the habits of the species of *Glossina* one is attacking; a single species can show wide variation in its habits under different circumstances and seasons.

During the course of the earlier trapping work which covered a variety of types of fly-belt, a useful and fairly comprehensive working knowledge of the desiderata for the successful siting of traps was obtained. Some of the more important conclusions have been tested by a series of experiments during the past six months. These experiments were conducted on the Black Volta, all groves now being cleared and free from fly. Two obvious feeding grounds of *G. palpalis* and *G. tachinoides* were chosen having a canoe crossing with abundant traffic, people constantly washing and drawing water, and cattle drinking. A fishing camp was adjacent to each. Only low fly-belt occurred in the immediate vicinity; high, dense fly-belt was about half a mile distant in each case. A third experimental site was in high, dense, undisturbed fly-belt with ample breeding grounds, the traps being placed in two natural openings, 14 yards and 100 yards in width, on the river bank. Human and domestic animal hosts were infrequent here but small mammals and reptiles were fairly plentiful. The sites were marked with numbered pegs and sets of 4 traps were moved round in a 4-weeks' rotation. The traps were emptied and their catches recorded every second day. To eliminate the effect of variation in efficiency between one trap and another the results are assessed as 4-week totals.

In most of the localities trapped, *G. tachinoides* was the predominant species and *G. palpalis* relatively rare. Wherever the latter has been caught in numbers sufficient to be significant it has followed the behaviour of *G. tachinoides* very closely in regard to site preferences. Moreover, as the whole object of the experiments is to contribute towards the control of human trypanosomiasis, in the transmission of which both species take part, feeding grounds where humans form the principal hosts have been chosen as the key points for trapping. Thus the two species, despite their different host preferences, can be considered together in the following observations on the siting of traps. It is quite possible that in places such as the moister southern savanna forest, or the true rain forest where *G. palpalis* is found predominant or alone, its siting requirements may be different. But the difference is more likely to be due to the influences of environment than to factors inherent in the species.

It will probably be most useful to the practical worker, and it will certainly make for clarity, if the results of this accumulated experience is presented under headings. What should not be overlooked is the concept of their close interlinking nor the fact that, according to circumstances, sometimes one factor, sometimes another, may have the dominant influence.

Factors for Successful Siting.

(*a*) *Environment.*

Animal traps have been found to be effective anywhere in the permanent habitats of the tsetse, provided that they are visible. They caught very well in the thickest type of fly-belt where *G. palpalis* and *G. tachinoides* are very numerous and breeding goes on regularly; but at the same time, slightly larger catches were being made in an open feeding ground, 1½ miles distant, where the tsetse incidence as measured by flyboys' catches was approximately three-fifths of that in the fly-belt. That is to say, a considerably greater proportion of the flies apparent to humans were being caught in a feeding ground than in undisturbed fly-belt. This is a point of great importance in the consideration of trapping as a means for the control of trypanosomiasis, and it is a consequence of the fact that, as will be seen below, feeding grounds present the greatest combination of factors favourable to trapping. It should be recognised, then, that although traps catch well anywhere in a fly-belt their most advantageous employment will always be in regular feeding grounds where man is the principal host. Such localities will be on open river banks at ferries, bridges and fords, at water-holes, washing places, or animals' drinking places wherever there is closely adjacent fly-belt. Typical sites of this open nature are shown in Pl. XXIV. In each case the fly-belt can be seen to be quite close. Paths along fly-infested rivers, either above the bank or below it, and along the actual water-course when this is dry, can also afford good trapping sites. But paths crossing the river are better, since they tend to concentrate the hungry flies at one point, where the path passes through the vegetation fringing the banks. In such places, for example Pl. XXIII, fig. 3, traps can be very effective. Traps have been found to catch quite well in short protective clearings which are regularly traversed by a certain number of hungry flies. In one mile-long clearing, traps consistently caught more flies than a team of flyboys during four or five months of the dry season for three years in succession. This indicates clearly the protective potentialities of traps.

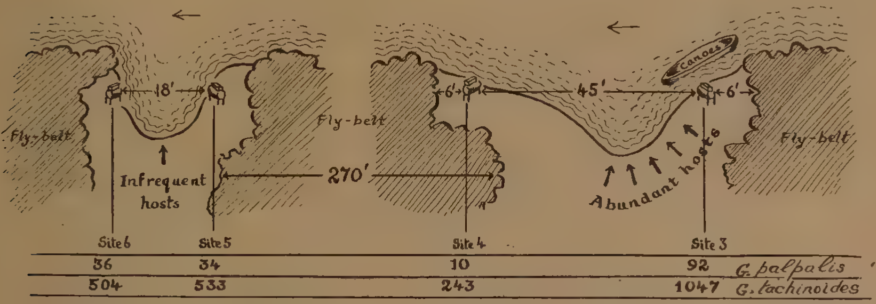


Fig. 2.—Influence of the presence of hosts on trapping. Diagram of two open feeding grounds, 90 yards apart, on the Black Volta river close to Lawra. The upper feeding ground at the canoe crossing had all-day presence of people and animals. The lower opening had daily visits from a few people only and occasional animals drinking. The catches of *G. palpalis* and *G. tachinoides* in the four trapping sites are totals for a period of four months.

(*b*) *Presence of Hosts.*

The regular presence of hosts in considerable abundance seems to be the most important single factor for prolific trapping. Furthermore, the closer the trap can be placed to the real hosts the higher will be the catches. The only limit to this intimacy is that in the best position, right among the hosts, the trap is liable to get upset or pushed aside.

At the two canoe crossings of the recent siting experiments, the relationship of hosts to trapping efficiency was brought out unmistakably. The sites in one of these feeding grounds, together with their respective fly catches for the 4-month period April–July, 1947, are shown diagramatically in fig. 2 and appear in the photograph (Pl. XXIV, fig. 3). Site 3, occupied by the trap at the right of the photograph, was right alongside the spot where the canoes land and where people are constantly drawing water, washing clothes and bathing, the nearest patch of fly-belt being only 2 yards away. Traps in this site caught an aggregate of 4 times as many flies as those in site 4, only 15 yards away and equally close to fly-belt, but on the extreme edge of the opening and 8 or 9 yards from the regular human and animal traffic. Sites 5 and 6 were in a similar, though narrower, opening 90 yards downstream, but with no canoe landing place and only occasional people and animals visiting the waterside. The traps here were placed in exactly the same relation to fly-belt and the water's edge as those in sites 3 and 4, yet they caught only half as well as those in the presence of frequent and plentiful hosts. Almost the same degree of influence of abundance and rarity of hosts was found in a four-months' trial at the second canoe-crossing which was immediately below a fishermen's camp. In the best position, right in the open and exactly at the canoe landing place, 726 *G. tachinoides* were caught during the trial. In another position 12 yards away and close to fly-belt, but not so close to people, only 393 *G. tachinoides* were captured. In a similar opening 25 yards off, with no canoe landing place and infrequent visits from people, two sites in an exactly similar relation to fly-belt accounted for 451 and 342 flies.

In short, a feeding ground with abundant hosts can produce double the catches where hosts are infrequent. Sites in the closest possible proximity to hosts can give 2–5 times the catches made in a site 9 or 10 yards away.

With the game tsetse in E. Africa the effect of the presence of hosts in heightening catches has been frequently observed. Dr. R. du Toit and Dr. E. B. Kluge find that, in low concentrations of *G. pallidipes,* Harris traps cease catching effectively unless cattle are driven up to the vicinity of the trap (personal communication).

It has frequently been observed by the writers that humans and cattle coming to the waterside soon collect a swarm of hungry and interested *G. palpalis* and *G. tachinoides,* and the swarm tends to increase as long as the people linger. When the hosts depart, the traps are the most conspicuous objects remaining and it is then that the flies are most likely to be attracted to the traps and captured. Further, it has been observed that when humans and traps are together both species of *Glossina* tend to go to the people first, and only if repeatedly driven off will they go to the traps. From the rapidity with which one is attacked at well-frequented river crossings and water holes, it seems possible that frequency of hosts induces a regular habit in the flies of frequenting such places, to the neglect of other feeding grounds to all appearances equally promising but without the same frequency of hosts.

(*c*) *Relation to the Tsetse's Movement.*

Tsetse of the *G. palpalis* group tend to follow definite routes which remain fairly constant as long as there is no marked alteration in the environment. These routes are almost impossible to describe but they can be recognised with experience and especially with the help of expert flyboys.

It is always an advantage to place traps between the fly-belt and the area frequented by hosts so as to intercept the hungry flies on their way to feed; and if the route can be determined exactly and traps placed on it, they will catch better than those placed a little to one side. A good example of this occurred at the feeding ground pictured in Pl. XXIV, fig 2, which is at the edge of Zambor Grove, the location of prolonged trapping experiments.

A small Swynnerton's A.S.B. trap was placed directly in the route taken by flies from the dense fly-belt seen in the background along the little gully to the water-hole, where the women were wont to foregather. This particular site was far superior to sites on either side of the water-hole, such as that occupied by the black Animal trap on the right of the picture, and it was also better than positions in the higher ground along the edge of the fly-belt although traps there appeared equally conspicuous. As the A.S.B. trap caught so well it was left in this site for 12 months. During that time its catch equalled the average catch of seven Animal traps scattered through the groves and it was only a third less than catches made by the two best-sited Animal traps. Yet general long-term comparisons show that the Animal trap has 2½ times the catching efficiency of the

A.S.B. trap under grove conditions and 9 times the effectiveness in the open. The site occupied by this particular screen trap can thus be said to have been at least 2½ times better than a variety of neighbouring sites.

In the next grove, Yahtori, two of the consistently best sites were right in the bed of the watercourse where sand-spits made possible the placing of traps. These traps were not on regular footpaths; they were not in a place where hosts collected and, with complete overhead shade, were under poor lighting conditions. But they were both on the direct route followed by the tsetse along the stream bed, and this factor made up for the other apparent disadvantages of site.

(*d*) *Visibility.*

A trap must be as obvious as possible. In particular, it must be readily visible from those parts of adjacent fly-belt where hungry tsetse are known to linger. Consequently the greater the angle of visibility of the trap the more tsetse it will attract. A background which shows up the trap by contrast is an advantage. It is this factor of visibility which enhances the value of open stretches of river bank and especially of sand-spits and promontories jetting out into the river, and of the convex bank at bends, the more acute the better (Pl. XXIV, fig. 1).

In the siting of a trap in open ground there is a certain range of distances from the edge of the nearest fly-belt within which maximum catches are made. The optimum distance will vary according to local conditions and possibly also to season, but extremes either way, too close to the bush or too far off, decrease the catches. Trials in a natural opening in dense riverine fly-belt, where there were no definite host routes to bring in complicating factors showed that a site 5 yards out from the fly-belt caught nearly twice as well as a site on the other side of the opening in which the trap stood right up against the edge of the thicket. In an 8-months' trial the two sites caught 107 *G. palpalis*, 1,104 *G. tachinoides*, and 34 *G. palpalis*, 652 *G. tachinoides*, respectively. Again, in a second opening 100 yards in length, a site 6 yards from the fly-belt edge caught 1½ times as many flies as a site 48 yards out, the respective catches during the 8-months' trial being, at 6 yards 96 *G. palpalis* and 1,111 *G. tachinoides*, and at 48 yards 48 *G. palpalis* and 706 *G. tachinoides*. In the latter case there was no regular attendance of hosts to attract the flies particularly to the middle of the clearing. The similarity of the catches at 5 yards and 6 yards from fly-belt in these two openings, which were separated by 200 yards of bush, is very striking.

It is probable that in these experiments and in many similar observations, limited visibility explains the falling off in catches with traps placed too close to fly-belt, proximity reducing the periphery of the flies' habitat from which the trap is easily visible. In the case of distance lowering the catches, the explanation may be that the traps are beyond the range at which tsetse are visually attracted or it may be that only the very hungry flies are tempted out to such a distance. The proximity of hosts will greatly increase catches in traps set right out in the open, partly by adding the extra attractions of movement and scent and also by the movements of the hosts, which will occasionally bring them close to the fly-belt and thus stimulate the flies to follow them back into the open.

(*e*) *Orientation.*

Bearing on the factors of visibility and movement, the orientation of the trap with its long axis at right-angles to the usual line of movement of the tsetse is of great importance. If the flies' line of movement is direct from fly-belt to host then the traps should always be placed across this line, as in Pl. XXIV, figs. 2 and 3. At rivers and streams a regular movement of flies goes on along the water's edge, so positions with the traps at right-angles to the edge of the water and as close to it as possible are often satisfactory. This type of siting, however, often involves the risk of losing traps through rising water and floods.

(*f*) *Lighting.*

Good lighting is almost inseparable from good visibility. Traps in the open always have good lighting. Those placed within the fly-belt should have at least good top lighting; if possible direct sunlight should reach the trap. This throws the body of the trap into good relief, making the most of its attractiveness, and it also ensures a good light through the gauze catching cage which draws the flies upwards from the dark interior of the body. This lighting condition would seem essential for the successful working of a trap. Surprisingly enough it is by no means so. In the sacred groves some of the consistently best sites were completely under canopy with only filtered light reaching the traps from above, but each was in a clear open space in which it stood out obviously, and was placed either directly in a fly route or at a spot where concentrations of flies could usually be found.

Finally, there is the question of the consistency of sites. Good sites, the determination of which is essentially a matter of experience, observation and trial, remain good as long as there is no radical alteration in the environment. Bad sites remain consistently so. Frequently, when traps are put into new sites they make initially high catches which may last for a few days or even a week or two, after which there will be a considerable drop in the numbers of tsetses taken. This phenomenon has been observed wherever trapping experiments have been started in previously undisturbed fly-belt. In three recent experiments the catches of groups of 4 traps kept in the same positions fell by 30, 50 and 75 per cent. in the course of the first month after which they become fairly constant. But the same thing will happen in the case of flyboys catching in a new locality, and it marks an effect on the fly population rather than a diminution in the effectiveness of the traps. It probably represents a skimming off of a part of the population in the immediate environment of the traps. The steady catches that follow are thus, in a sense, a rough measure of the replacement rate of that part of the population by immigrants from outside the range of the traps and by new emergences. Traps have been kept under observation in the same sites for several years during which time their catches showed considerable fluctuation according to the flies' abundance and the favourability of conditions for trapping. It is at the season of peak catches that good sites show to advantage. They may yield two or three hundred flies to the twos and threes from poor sites and their catches at such times may be twenty times as great as when conditions are least favourable. In a limited environment the numbers of flies caught by traps may show a decline which continues for longer than would be expected as the result of initial skim-off of population or of seasonal change. In such a case the traps are having a direct effect in reducing the fly population. This aspect will be discussed more fully under "Control."

Comparison of Traps with Flyboys.

(*a*) *Method of Comparison.*

In making estimates of tsetse incidence the majority of workers adopt hand-catching by flyboys as the simplest and quickest means of obtaining information on the presence or absence of the various species and a general idea of their relative abundance in different localities and at different seasons. It has, of course, been well recognised that flyboy catches do not by themselves represent the true density of the flies and that they merely give a measure of the density-activity complex in so far as it happens to be modified by the climatic conditions prevailing at the time. When flyboy catches are made over an extended period in one place, the numbers of flies caught tend to show a seasonal rhythm, and this rhythm can usually be interpreted in a way that fits in logically with other information on the biology and ecology of the various species of tsetse. Such interpretations provide useful working hypotheses and are often the foundation of ideas which may eventually approach more closely to ultimate truth; and the continuation of the observations on which they are based is thus justified, provided that the observer remains aware of the limitations of his methods. One obvious use of routine catching by flyboys is to test the efficacy of attempts at controlling tsetse, for, although the numbers of flies caught may not be strictly proportional to the fly population, they will at least show whether or not the fly colonies have been substantially reduced. As the writers were familiar with the seasonal rhythm of flyboy catches in the various types of fly-belt in the district, it was appropriate during the course of the trapping investigations to make parallel series of hand-catching records for the purposes of comparison. This has given some unexpected results, the full implication of which is not yet fully understood and forming the subject of continued study.

The routine flyboy catches are made by teams, usually of four boys, who visit each locality for a period of 4–6 days every month and catch flies from 7 a.m. till 3 p.m. daily. The numbers of flies caught are then standardised to a catch of 20 flyboy-days a month for each place. In the first attempts to compare trap efficiency with flyboy efficiency, the trap catches also were standardised to 20 trap-days a month, but the resulting figures were such small fractions of the flyboy figures that significant variations were lost. It was then decided that, as the flyboy standardised catch had been chosen because it closely represented the actual catch, it would be better to select a trapping unit which had some bearing on the work done per month by the traps in each place. For practical and economic reasons, too, this seemed a more rational way to make the comparison, because it is not, as a rule, feasible to allow more than 20 flyboy-days a month for a routine observation at one place, whereas one would almost certainly put more than one trap at an observation point, and all the traps, apart from possible brief spells of inactivity due to damage, would be in operation for the entire month, 24 hours a day. The trapping unit chosen was 5 traps working for 30 days, i.e., 150 trap-days a month. In practice there were generally 4 – 7 Animal traps working in each locality and the actual number was very often 5. Higher numbers were used only for special purposes, so that the range of catches chosen for particular study suffered very little modification from standardisation.

(*b*) *The Differences in Reaction according to Species, Sex and Environment.*

The comparison of trap catches with flyboy catches has revealed certain differences in the flies' reactions to the human and artificial attractants. Some of these differences are striking; probably all are important in that they stimulate the attention into a more critical analysis of the observed phenomena.

Table I summarises certain of the fly catches made by Animal traps and teams of flyboys during 1942. In each case the catch represents an unbroken series of twelve monthly records standardised according to the convention described. The Volta-side trapping place was at a much-frequented canoe crossing, where, also, the people of the near-by village of Baghari come to wash, draw water and water their stock. At the crossing there is a natural open clearing about 30 yards wide. On each side of this the vegetation is mainly low, dense *Mimosa asperata* and *Quisqualis indica* with occasional *Pterocarpus santalinoides*, and the typical Volta-side fringing forest of *Cola laurifolia*—*P. santalinoides*—*Garcinia baikeana* associations do not occur within half mile of the crossing. The traps were sited on the open river banks at the crossing. The flyboys were free to search along the river banks within 100 yards of the crossing. At Kamba Bridge the main road crosses a principal tributary of the Volta. In 1942 the roadway was carried 12 ft. above the river bed on a bridge supported by innumerable tree trunks. As the river banks had been cleared for some distance on each side of the road the bridge itself provided a shady resting place for tsetse on the look-out for hosts. In the wet season, when the river was full, the traps were set on top of the banks but, as the water receded in the dry season, some were moved down to the actual river bed. In the dry season of 1941–42 selective clearing operations were in progress on the Kamoa (Morris, 1946), and at the bridge *G. tachinoides* was reduced to about one-fifth of its former incidence. Observations were made in four groves situated several miles apart along the much smaller Lawra stream. The groves themselves were not cleared until three years later, but in 1942 there was no connecting fly-belt between them. Their individual fly populations were relatively small and so the figures quoted are the aggregates for the four, the data from each being first of all standardised to 20 flyboy-days and 150 trap-days a month.

TABLE I.

Relation of Trap Catches (5 traps) to Flyboy Catches (20 Flyboy-days a Month), based on monthly catch Figures for 1942.

	Total flies caught						Percentage of females in catches		Trap flies/ 100 flyboy flies		
	Traps			Boys							
	♂♂	♀♀	Total	♂♂	♀♀	Total	Traps	Boys	♂♂	♀♀	Total
G. tachinoides											
Volta-side Baghari	3,168	1,737	4,905	1,401	2,178	3,579	35	61	226	80	137
Kamba Bridge ...	160	111	271	71	115	186	41	62	227	96	146
Groves (sum of catches in four groves) ...	493	405	898	299	229	528	45	43	165	177	170
G. palpalis											
Groves	223	242	465	424	400	824	52	49	53	61	56
G. morsitans											
Kamba Bridge ...	272	235	507	471	312	783	46	40	58	75	65

The first conclusion to be drawn from the table is that the various species of tsetse differ in their relative response to flyboys and traps. Flyboys working for 20 flyboy-days a month in each grove caught altogether in the four groves during the year 824 *G. palpalis* and 528 *G. tachinoides*, while the traps, at the rate of 5 traps in continuous operation in each grove, took 465 *G. palpalis* and 898 *G. tachinoides*. That is to say, the group of traps relative to the team of flyboys is more efficient in the case of *G. tachinoides* or, alternatively, less efficient for *G. palpalis*. This was explained by the observation that, although both *G. palpalis* and *G. tachinoides* feed on man and animals, the former has a marked preference for man and therefore comes more readily to the flyboys, whereas the latter is more catholic in its feeding habits and so is more likely to be deceived by the traps' imitation of one of its customary hosts. Thus, of the available tsetse, a higher proportion of the *G. tachinoides* than of the *G. palpalis* would be likely to enter the traps.

At Kamba Bridge flyboys caught 186 *G. tachinoides* and 783 *G. morsitans* while the traps caught 271 *G. tachinoides* and 507 *G. morsitans*, i.e., relative to the flyboys the efficiency of Animal traps for *G. morsitans* is much the same as it is for *G. palpalis*. As *G. morsitans* is the tsetse of game animals it might be expected that any success of the Animal trap which resulted from its resemblance to an animal would be most evident in the case of this species. However, as *G. morsitans* in this part of West Africa is essentially associated with the presence of the larger game animals such as buffalo, roan and hartebeest, the small size of the animal traps may have been unfavourable. Movement, also, is of great importance in attracting *G. morsitans*, which is notorious for its habit of following not only its natural hosts but any sizeable moving object such as a motor-car or a cyclist. East African workers realised the importance of movement in their attempts to attract *G. morsitans* to traps, and they made traps to swing in the wind, revolve, or even travel to and fro suspended from a wire. The present workers, on the other hand, have at no time been concerned with the possibility of controlling *G. morsitans* in West Africa by means of traps. But although the catching of *G. morsitans* at Kamba Bridge was quite fortuitous, the results are of sufficient interest to be worth recording.

The next point to be considered is the proportion of female flies found in the different catches. The great diversity of the ratio between males and females in captures of tsetse is a well-known phenomenon and has been the subject of much discussion. Explanations have been given of the causes of variation in the sex ratio as effected by different types of environment and changes in the season and, in general, of how these factors affect different species. The data under consideration represent catches made uniformly during a period of 12 months, i.e., a complete seasonal cycle, thus presumably eliminating variation due to time of year. In the case of *G. tachinoides* at the Volta, there is a very wide difference between the percentage of females in the boys' catch and that in the trap catch, so great, in fact, that, although the group of 5 traps proved to be on the whole superior to the team of flyboys working at intervals it was the boys who caught the greater number of females. The same was true at Kamba Bridge. In both cases the superiority of the traps lay entirely in their relatively greater attraction for males. In the groves, on the other hand, the proportion of females in the trap catches was higher, though not significantly higher, than that in the flyboy catches; in other words, there was no differential reaction on the part of the male and female *G. tachinoides* and the superiority of the traps was absolute. This difference can be satisfactorily explained as being due to environment. A grove is a small area and, by its very nature, the breeding places and feeding places are close together or even identical. At Baghari and Kamba Bridge the traps were set in definite and very open feeding grounds, which were well removed from regular breeding sites, at the former place by more than half a mile, at the latter by 5 miles of clearing. The assumption is that, while both males and females visit feeding grounds for the purpose of feeding, males anxious to mate wait around the food hosts in the hope of meeting females. The Animal trap, coming within the category of preferred hosts of *G. tachinoides*, deceives the non-hungry exploring males as well as hungry flies of both sexes. Flyboys catch the flies that settle on them, but, in addition, and in contrast to the traps, they move about looking for flies to catch; good boys are expert at locating the kind of shady spot where flies like to rest. Any searching along the river bank away from the open feeding ground automatically takes the boys nearer to the denser fly-belt of the breeding sites, and so it would be natural that their catches should contain a higher proportion of females than the trap catches; although why the difference should be so great, particularly at Baghari, is not clear. In the groves, as there would be much less differential distribution of the sexes and no difference between the types of terrain covered by flyboys and traps, it would also be natural that the sex ratio in the two sets of catches should be similar.

It might be expected that the above argument, if valid, should also hold good in the case of *G. palpalis*. The difficulty is that in comparing the efficiencies of traps and flyboys there is no absolute standard. Deductions can be made about the reactions of *G. tachinoides* by comparing the happenings in different environments. A conclusion about *G. palpalis* can be derived only from comparison with *G. tachinoides*. The following statements are therefore presented as a provisional hypothesis only. As has been said above, *G. palpalis* is more closely associated with man than is *G. tachinoides.* It is thus understandable that, in comparison with the flyboys, Animal traps should be less efficient in respect of *G. palpalis* than with *G. tachinoides*. Why then should the proportion of female *G. palpalis* be higher in the trap catches than in the flyboy catches? In the small space of a grove one flyboy would quite likely move about looking for flies, but with a team of four each would have little reason to move far from his chosen site. They thus become part of the group of food hosts which are attended by males waiting for females. Since humans are preferred to animals by *G. palpalis,* it follows that males are much more likely to linger round real humans than round dummy animals; so the reason for the difference in the sex ratios is that the boys attract and therefore capture a higher proportion of males.

The relationship of *G. morsitans* and *G. tachinoides* in catches by flyboys and traps and the difference between the sex ratios of *G. morsitans* in the two catches are rather similar to those found in the case of *G. palpalis*; but as the habits of *G. morsitans* are quite different from those of *G. palpalis* and the environment is also different another explanation must be offered. *G. morsitans,* although having game animals as its principal hosts, prefers, as has been seen, a moving human to a stationary animal. As before, some feeding males and females go to the traps and are caught; some feeding males and females go to the flyboys and are caught. The males which are merely waiting for females tend to congregate round the preferred moving hosts so that altogether the proportion of males would be higher in flyboy catches than in trap catches. As the breeding places of *G. morsitans* are widely distributed throughout the bush and are in no way associated with the fly-belt vegetation of *G. palpalis* and *G. tachinoides,* the argument, which explained why a flyboy catching *C. tachinoides* in a clearing would tend to catch a higher proportion of females if he moved towards the end of the clearing, does not apply at all.

In brief, it is reasonable to assume that the influence of traps on the sex ratio of the GLOSSINAE is closely related to the host preferences of the species concerned. In the case of *G. tachinoides* the Animal traps represent a class of regular hosts and so attract attendant non-feeding males as well as hungry flies of both sexes. In the case of both *G. palpalis* and *G. morsitans,* the traps represent something outside the range of preferred hosts and therefore are ignored by attendant males and investigated only by hungry flies intent on obtaining a meal off the first available host. When samples of flies include many hungry individuals the proportion of females is always high. In East Africa it has been recognised that trapping meets with the greatest success when the fly proportion is particularly hungry (Swynnerton, 1933), and Lloyd considers that *G. palpalis* enters traps (Swynnerton's A.S. pattern) only when hungry and that its reaction to traps is definitely a host reaction (Lloyd, 1940).

Finally, the effect of environment on the efficiency of Animal traps relative to flyboys is worth noting. Although the comparisons can be made only with reference to *G. tachinoides* it is fortunate that this species has been captured in all three situations. We have expressed the efficiency of traps relative to flyboys as the ratio :—Number of flies caught by traps per 100 flies caught by flyboys, i.e., Trap Flies/100 Flyboy Flies. This ratio is lower on the Volta, where the fly population is dense and very extensive and not at all likely to be affected by the removal of the samples captured. It is highest in the groves where the fly populations are small and to a large extent isolated.

It is well known that the presence of humans or animals in the vicinity of traps results in increased numbers of flies being taken in the traps. Tsetse find their hosts by sight and, to a lesser extent it is thought, by smell. The presence of moving things round the inanimate bait would thus form a visual attractant, much more obvious from a distance, and would at the same time provide a stimulus to the flies' olfactory sense. Since the object of the flies is to feed, it follows that the greatest success of traps will result from the presence of a sufficiency of real hosts to focus the attention of as many flies as possible in the environment, but not such a large number as to minimise the chance of a fly coming into contact with a trap rather than with a real host. A tsetse has a natural and complete reaction to its true hosts and this instinct includes a tendency to prefer one species to another. Since tsetse are in fact caught in traps it appears that they are liable to be deceived up to a point. The writers, wishing to have direct observations of the flies' reactions to traps, set sharp-eyed boys to record what took place. The only positive result of this experiment was that if a boy sat close enough to a trap to be able to see the flies on it the tsetses, *G. tachinoides* as well as *G. palpalis,* invariably preferred the real boy to the imitation goat and so would not alight on the trap. In the ordinary sense people and animals do not compete with traps at a trapping site. Their blood may satisfy the hungry fly for the time being, but the fly is not eliminated for it is much too agile to suffer from the hasty slap or flicking tail which is the almost unconscious reaction of creatures long inured to the presence of annoying insects. An experienced flyboy, on the other hand, accounts for a high proportion of the tsetse which come his way and he is likely to intercept a number of flies, which, but for his presence, would eventually have found their way into a trap. When a flyboy sees a fly on his own leg, or elsewhere, he concentrates on keeping quite still, giving it a sense of security until the instant of its capture. Ordinary people and animals irritated by the bites, keep the flies on the move; and this may well be an additional reason why a tsetse, landing on a less attractive but entirely passive trap, lingers there undisturbed long enough to explore and so get caught.

The Baghari trap records were examined in detail to see whether the catching powers of the traps tended to be reduced on the days when the flyboys were catching in competition with them, but this did not seem to be the case. Nor would it be expected that the presence of flyboys would cause the traps to appear less attractive to the flies in an environment where numbers of humans are already constantly present. As there is at all times a variation in the numbers of flies taken daily in a group of traps the influence of the flyboys would have to be considerable in order to be evident. The figures for Kamba Bridge and the groves are too small to be valid for detailed analysis, but it might well be that with the very much smaller numbers of flies available the traps would be catching fewer flies on the days when flyboys were present. For the same reason, had no traps been present, the flyboys would also be catching fewer flies. Now the figure for 20 flyboy-days is based on the work of 4 boys for 5 days, at a time when all the traps were in continuous operation. But the figure for 150 trap-days is derived from the work done by 5 trap days on 5 days, during which the catch is reduced by competition, plus the work done on 25 days with no competition. The trap catches and flyboy catches at Kamba Bridge and in the groves are therefore less strictly comparable than they are at Baghari where there is virtually no competition, and so it is not surprising that the traps appear to be more efficient in the groves.

(c) *Seasonal Variation.*

The most striking feature that emerged from the comparison of trap catches with flyboy catches was the great difference in their annual rhythms. Flyboy catches of *G. palpalis* and *G. tachinoides* in this region form characteristically a double-peaked curve. The absolute minimum occurs during the dry season and has been interpreted as being due to a low population restricted in environment and frequently much reduced in activity because of the harmattan. There follows a rapid rise to a maximum in the early rains, a real increase in population, then a drop succeeded by a second and usually lower maximum, indicating a high population distributed through all the wet-season habitats. The actual times and the relative values of the maxima and minima vary considerably according to the type of locality in which the collections of flies are made. They are also influenced by the climatic conditions of the year in general and by the weather immediately preceding and during the period when the flyboys are working. But in spite of the irregularities in graphs drawn from the data, the trends are usually recognisable to anyone who is familiar with the concept. In contrast the trap catches show a definite minimum in the wet season and a maximum during or towards the end of the dry season. Less determinate variations occur between the maximum and the minimum. In fig. 3 the graphs, which show the catches made by boys and traps during two years at Baghari on the Volta, illustrate this typically, especially the irregularities, and they show little more than that the traps function relatively well in the dry season and relatively badly in the wet season. In the histograms, however, the monthly catches have been smoothed in threes and the resulting simplification of outline emphasises that the seasonal variations of trap and flyboy catches are quite distinct from one another.

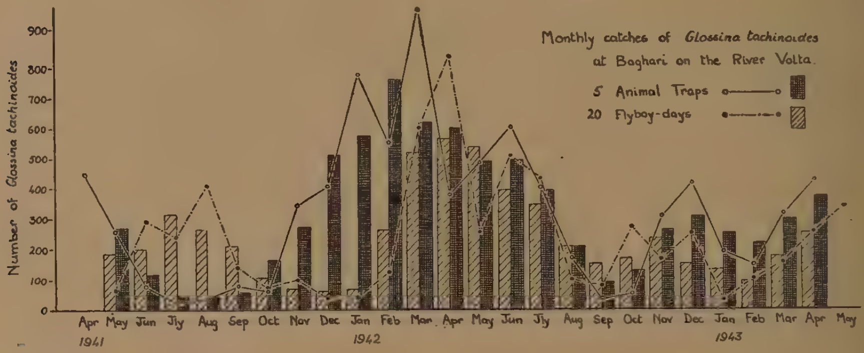


Fig. 3.—Seasonal variations in catches of *Glossina tachinoides* made by flyboys and Animal traps on the Volta at Baghari. In the histograms the monthly catches are smoothed in threes.

When flyboys are making routine monthly catches at a number of places, it is usually more convenient for the boys if they put in their quota of days consecutively at each place. For long-term observations to be dealt with in a broad sense this method works quite well, but it has an obvious disadvantage if, later, the same data are to be utilised for detailed comparisons. For example, suppose the team visits place A in the first week of July; there is no rain during the period and the weather is, on the whole, sunny with little wind. The following week the team is at B and it experiences two heavy rain storms accompanied by wind, the sky being overcast every day. The difference between the catches made at A and B represents the combined effect of environment and weather, and to assess the factors separately is not a simple matter. Again, one expects catches made in December to be less than similar catches made at the same place in November, because the harmattan, when it blows strongly, inhibits fly activity. But the team may happen to pay its visit during a windy spell in the usually stuffy November and during a quiet spell in December, so that the results are reversed. A group of traps operating all the time should not be subject to the same eccentric variations, but even in their case the bi-weekly records show great divergencies in the numbers of flies taken. The trap catches would be expected to give a better measure of the effect of the month's weather as a whole in so far as it influences the flies reaction to traps. At the same time the traps depend also on the presence of real hosts, human and animal, and the variation of this factor was not recorded in the data under review. As fig. 3

shows, a very simple way of overriding these difficulties is to smooth the figures, but this method has the disadvantage that as a foundation for explanations or theories it is too artificial.

TABLE II.

Animal trap catches standardised to 150 trap-days a month and flyboy catches to 20 flyboy-days a month.

	Total *G. tachinoides* caught in all localities		Percentage of females in catches		Relative efficiency of traps and flyboys					
					Trap flies per 100 flyboy flies			Percentage in traps of sum of traps and flyboy catches		
	In traps	By fly-boys	Traps	Fly-boys	♂♂	♀♀	Total	♂♂	♀♀	Total
January	1,173	109	33	46	1,339	766	1,076	93	88	91
February	994	287	37	48	422	265	346	81	73	78
March	2,043	1,125	43	56	238	138	182	70	58	64
April	1,785	1,459	45	63	179	88	122	64	47	55
May	1,042	567	46	54	209	162	184	68	62	65
June	918	938	49	57	117	83	98	54	45	49
July	1,494	1,709	47	59	114	69	87	52	41	47
August	485	854	50	54	63	52	57	39	34	36
September	482	2,008	43	66	40	16	24	29	14	19
October	522	1,125	58	66	56	41	46	34	29	32
November	940	725	44	56	166	101	130	62	50	57
December	1,096	542	36	50	259	146	202	72	59	67

It was because of the natural irregularities that the first inspections of series of trap and flyboy catches did not reveal the full extent of the differences between the two. When, however, all the standardised parallel trap and flyboy catches of *G. tachinoides* were tabulated and summed according to months, they provided the information shown in Table II and fig. 4. Altogether the numbers represent catches made in 7 localities over a period of 4 years, but not in each place all the time, so that they cannot be used as a measure of seasonal abundance. In both trap and flyboy catches the percentage of females in the flies captured varies considerably during the year, being lowest in each case during the dry months and tending to be high during the wet season, but the flyboys caught a consistently higher percentage of females than the traps. The efficiency of the traps relative to the flyboys is measured by the same formula as before, viz., trap flies per 100 flyboy flies, and also by expressing the trap catches as a percentage of the total number of flies caught by the traps and boys together. Since, every month, the traps caught a lower percentage of females than the flyboys, it follows that at all times their efficiency relative to flyboys is higher for males than for females. The traps are at their highest efficiency in January and their lowest in September, the latter being only about one-fortieth of the former. This is in general agreement with the findings for *G. morsitans* which, in Tanganyika, was trapped in numbers that were considered worth while only in the hottest part of the dry season (Swynnerton, 1936), and the trap catches of which in Southern Rhodesia were negligible after the commencement of the rains (Jack, 1939).

If it were to be assumed that the flyboys' capacity for catching remained constant, or nearly so, throughout the year in relation to the fly population, then this peak of trapping efficiency in the dry season would be truly phenomenal. Jack's interpretation is that the fly's reaction to a trap at a distance is one of attraction to shade and that having reached the patch of shade the fly then has a tendency

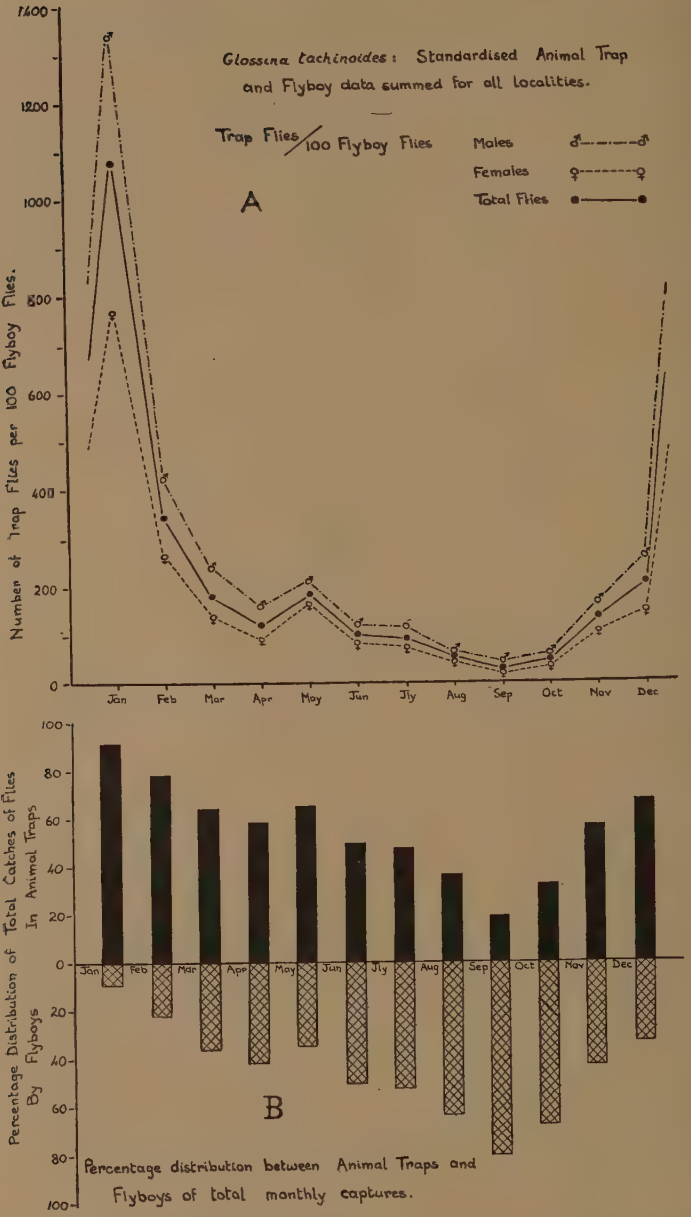


Fig. 4.—Diagrams illustrating the efficiency of Animal traps relative to Flyboys throughout the year and based on the observations from all places where Animal traps and Flyboys were working simultaneously. The monthly catches for each locality are standardised to 150 Trap-days and 30 Flyboy-days and are then summed.

to penetrate into a dark interior should one be conveniently available. Jack designed his traps on this principle, and his contention is that the success of traps with *G. morsitans* in hot, dry weather is due to the effect of loss of water in accentuating the shade-seeking reaction of the flies at a time when natural shade is at a minimum (Jack, 1939 and 1944). This theory, however, does not, in the writers' opinion, explain their findings with the tsetse group of *G. palpalis*. It will be recalled that, in the present work, traps covered with natural hessian were, on the whole, more successful than black traps, and that this is thought to be due to their greater visibility against a shady background. The primary need for food is considered to be a more likely reaction on the part of the tsetse than that it should fly to the underside of an animal or a trap mainly because convenient shade is offered. As has already been pointed out, some of the best trapping sites in the groves were under complete canopy where additional shade appeared to have no value at all. If sunlight did in fact reach these traps, causing shadow effects, it was only for a very short period daily, whereas much heavier shade was available in the surroundings all the time. The consistent feature of these positions was that they were sufficiently open from the sides to make the trap a conspicuous object.

The traps show their highest catches in relation to flyboys at the period of the dry season when the harmattan is blowing most strongly and most regularly. The effect of this cool, dry wind is to cause an almost complete cessation of activity which may result in the flyboy team recording as few as ten flies for a day's collecting in a locality where tsetse are known to be abundant. Moreover, in the regular fly-rounds, from which all this information was obtained, the day's work ended at 3 p.m. when there is often a revival of fly activity, the temperature being higher after a long, cool and windy spell in the morning. This puts the flyboys at a great disadvantage since the traps remain to benefit fully from the afternoon activity, with no competition. Also the traps have the benefit of every lull in the harmattan when the flies, hungry because of low humidities and long periods of enforced abstinence, show considerable bursts of activity in seeking for hosts. At this time, too, the flies' habitat is severely restricted, its zone extending perhaps only ten yards from the water's edge. This helps to offset one of the disadvantages of traps, that they are fixed and dependent on the flies making contact with them instead of their being able to seek out and contact the flies.

But with the onset of the wet season, in April or May, the flies' potential habitat is considerably extended both by the increased atmospheric humidity and by the additional leafy cover of the deciduous vegetation. This extension of habitat does not immediately relate to breeding but it provides a much greater number of resting places from which flies can attack their hosts. The advantage of restricted distribution is now lost to the traps, and a falling-off is apparent in their efficiency relative to the flyboys who are free to move about and can locate temporary resting places. The extent of the advantage that has now been lost can be realised from a consideration of the importance of siting traps. In sites only ten or so yards apart the catches from a good site may be three or four times as great as from the others. From this it seems probable that the range of attractiveness of a trap is quite limited. When the width of the zone of activity of tsetse population is extended from ten yards to a hundred yards, the probability of contacts between flies and traps is proportionately diminished. As the wet season progresses the extension of the tsetse's habitat continues and secondary wet-season breeding foci are set up in thickets, termite-mound clumps, etc., away from the primary river-bank habitat. These can be visited by the flyboys but are out of the limited range of the traps which are kept as near as the rising water will permit to their original sites at the canoe landing-place, water-holes, etc. Thus, extension of the flies' range reaches its maximum towards the end of the rains in September-October, and it can be seen from figure 4 how the relative efficiency of the traps diminishes fairly steadily throughout the wet season to its minimum in September.

Two other factors operate to the disadvantage of traps during the wet season. By June or July supplies of water become available closer to the villages, causing the abandonment of the river bank for drawing water and washing. This breaks up the intimate association of hosts and tsetse that is maintained during the dry season to the great advantage of the traps, and some dispersal of the flies is the inevitable sequel. At the same time there is the loss of visibility resulting from the growth of herbage. Although a cleared space is maintained around each trap, the contrast between their visibility in the dry season, with bare open terrain, and the time when the grass and annuals have grown about three feet high, is extreme. It must be remembered that the traps possess only the visual attractions of colour and form, while boys attract by movement and scent also.

After the cessation of the rains, in November or early December, the visibility is restored by leaf-fall, by grass burning and by the trampling down of the herbage by people and animals. About the same time, or a little earlier, the abrupt transition from the tolerable climate of the rains to the adverse temperatures and humidities of the dry season causes the flies to become more and more restricted to their primary habitat. This restores the intimate contact between flies and traps essential to their maximum effectiveness and so in November and December a great increase in the relative efficiency of the traps occurs, finally bringing it back to its dry-season peak. This return to high efficiency is fairly abrupt, in contrast to the gradual falling-off during the onset of the rains; it reflects the rapid and complete change in climatic conditions.

Although seasonal rhythms of trap catches and flyboy catches differ widely from one another, each in its own way gives a representation of population variation combined with the effect of a changing season. Information is not yet adequate for the assessment of the several factors individually, but the traps have provided unmistakable evidence of a considerably greater fly population in the period December-February, the height of the dry season, than would appear from the catches of the flyboys.

Trapping for the Control of Sleeping Sickness.

The killing of adult tsetse as a prophylactic measure against sleeping sickness can be considered from three broad aspects. Firstly, under special circumstances such as occur in isolated fly-belts of limited extent, extermination of the flies might be effected, in which case control of the disease would be absolute. Secondly, it might be possible to reduce the tsetse population below the level at which it is significant for the transmission of trypanosomiasis. Thirdly, the continual destruction of flies that are feeding on people in places where the transmission of human trypanosomiasis is taking place will have a protective value in that every such fly is a potential vector and that, eventually, a considerable number of infected flies will have been destroyed.

(*a*) *Experimental Reduction of Small Tsetse Populations.*

It was in the sacred groves, with their apparently isolated and quite small populations of 100-500 flies, that the attainment of the first possibility seemed most likely. A method that failed here would certainly be ineffective in a more extensive environment. The possibility of exterminating such grove communities as the result of continuous catching by teams of 4 flyboys had been tested in 1939 in three groves in Lawra district. The results of this measure were essentially similar in each case and were apparent as a rapid decline in the numbers of both *G. palpalis* and *G. tachinoides* during the first few weeks, with the fly incidence lowered by 75 per cent. after a month's catching, followed by a more gradual reduction amounting to 90 to 95 per cent. after 8 weeks. Afterwards a solitary fly might be taken in the course of a week or two, then 5 or 6 together. This continued appearance might have been due to the emergence of flies, the groves being permanent breeding grounds. At the same time there existed the possibility of the introduction of flies from outside. It turned out that the groves in question were not beyond the limits of migration from neighbouring fly-belt, and this has subsequently been found to be the case for all groves in this part of the colony. Although a grove may be separated from the nearest fly-belt, which is often another grove, by a mile or more of open stream, the introduction of tsetse may occur at any time, either by the agency of their hosts, or spontaneously through the flies' habit of following watercourses. This latter movement is greatest in the rains when it is accentuated by a definite migration which has been known to extend as much as 10 miles up a river with no primary fly-belt. As the first experiments in catching-out groves had been carried out in the middle of the wet season, it was decided in 1943 to repeat the work at the end of the dry season when migration is not apparent but fly populations appear to be increasing. Of the four groves close to Lawra, which were the sites of trapping experiments, two were chosen. In these groves there had already been considerable interference with the fly populations through trapping, consequently the big initial drop in fly incidence which had taken place in the three initially undisturbed groves did not occur here. Instead there was a steady reduction in the numbers of both species of tsetse, and in the smaller grove, which had a light population, flies ceased to be caught after the fifth week when 90 *G. palpalis* and 26 *G. tachinoides* had been taken. In the larger grove the fly catches were reduced to nil after 12 weeks of continuous catching involving the destruction of 295 *G. palpalis* and 212 *G. tachinoides*. It was by this time mid-June. After 3 weeks without the capture of a single fly regular catching was discontinued but sampling catches were made for a period of one week each month. Tsetse of both species appeared in small numbers in August, in greater numbers in September, and a rapid increase took place in October. In the larger grove the incidence was now only slightly lower than that before the experiment was begun. In the small grove, which is closer to the primary fly-belt of the Volta, it was actually higher than before.

From these experiments it appears that fly communities which are completely isolated could be exterminated by the unremitting catching of adults by an adequate number of flyboys, a team of 4 being sufficient for the small populations concerned. Discontinuous catching, even at the rate of 30 flyboy-days a month, was useless as it failed to check the natural increase of fly. But completely isolated fly communities do not exist, and so to cope with introductions, especially those occurring in the wet season which can quickly regenerate the fly population, a team of flyboys would be needed to work continuously in each grove all the year round. Such a practice would be neither convenient nor economic.

Economically the employment of traps, with their cheapness of construction and simple maintenance, seems a more feasible method of tsetse control in groves. It would be possible to trap simultaneously in every grove along a stream and thus prevent the vitiation, by immigration from uncontrolled fly foci, of the results attained in individual groves. In 1940 continuous trapping was started in four sacred groves situated on the 10 miles of otherwise open stream close to Lawra. At first various types of traps were used but as the superiority of Animal traps became apparent these only were used, 5-7 bein maintained in each grove. In April, 1943, when trapping was discontinued in the two lower groves in favour of hand-catching, as described above, all the traps were transferred to the two upper groves. These groves, in each of which there were originally 7 traps, now contained 12 and 13. More traps were brought in from the Volta-side trapping sites and, after being reconditioned, were transferred to the two groves until, by July, the original numbers were more or less trebled. By then the groves seemed full of traps; it was difficult to find new sites more than five or six yards distant from existing traps and it was hard to imagine any tsetse in the grove not coming in contact with at least one trap during the day. It has been shown that the catching capacity of 5 traps for a month is roughly equivalent to the work of 20 flyboy-days, so that in each grove there would be needed 22 traps to represent continuous catching by 4 boys. By July this number was actually reached in one grove and 20 traps were operating in the second. Unfortunately this high concentration of traps could not be maintained. Difficulties in obtaining supplies, particularly of wire-gauze, nails and cloth, became acute and it was impossible to replace all traps as they fell into disrepair or were lost through flooding. However, 34-37 traps were kept in continuous use for the remaining 5 months of 1943. In the 10 months of 1944 during which the experiment was continued 15 traps, on the average, were kept working in each grove. By the end of that time the guardians of the groves had been persuaded to allow selective clearing to be carried out, and the measure was applied without delay since these fly-foci could not be allowed to persist indefinitely. Incidentally, it cost less than £20 to clear the 4 groves and the result was the immediate and permanent disappearance of both species of tsetse.

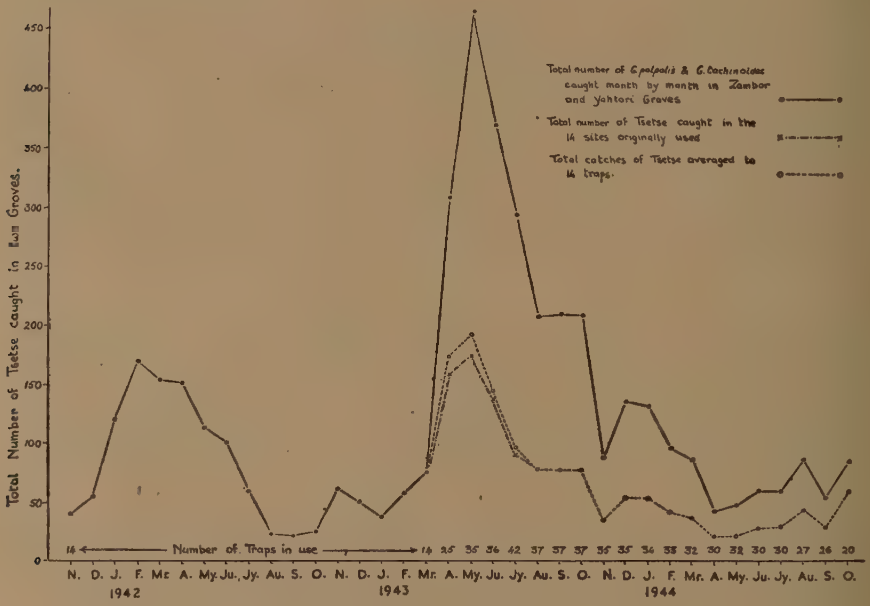


Fig. 5.—Record of an experiment on the reduction of isolated tsetse populations by intensive trapping.

Figure 5 illustrates the course of events before and after the additional traps were placed in the groves. Since the trends of the catches were similar in both groves and for both species of tsetse and, further, since both species are significant vectors of human trypanosomiasis and it is the practical value of trapping that is now under discussion, the representation of the data has been simplified to the total number of flies captured each month. This is shown by the unbroken line in the figure; the number of traps in operation is given for each month. From the beginning of 1942 until March 1943 there were seven traps in each grove occupying sites that had already proved to be good. These traps, or identical replacements, were kept in position throughout the experiment, new sites being found for additional traps. In the figure the broken line, starting at March 1943, shows the numbers of flies caught in those 14 original traps. The dotted line, on the other hand, represents the total number of flies caught each month corrected to an average of 14 traps.

The first thing to be noted from the diagram is that the seasonal rhythm of tsetse incidence, as revealed by trap catches, was not the same for 1943 as it was in 1942. The beginning of the rapid rise in the numbers of flies caught which was the feature of January and February, 1942, was in 1943 delayed until March. The second feature to be considered is the greatly increased number of flies caught as soon as additional traps were set out. Since the April catch by the 14 old traps was less than the 14-trap average of the total, it is apparent that the newly placed traps were having, at least for the time being, the advantage of previously untrapped sites. In May ten more traps brought about a further increase in the total catch which in that month reached its maximum, and there was an even greater difference in the average numbers of flies caught by the new traps and the old. By June the difference was no longer significant, showing that the advantage derived from placing traps in new sites was already wearing off. Also during this month the total catch of flies began a steep decline which continued at the same rate through July and August despite the addition of still more traps in July. The fact that this drop was arrested in September and October shows that wet-season, upstream migration was replacing the flies and compensating for the reduction of the fly communities by trapping. As far as can be judged the December peak following the November minimum corresponds to the December-January rise of 1942 and the February-March rise of 1943 but it is, of course, impossible to say what the catches would have been had only seven traps been operating in each grove. Although in this discussion the 1942 catches have been considered as representing the susceptibility to trapping of flies in the groves and not as an attempt at tsetse control, the destruction of these flies must nevertheless have had at least some influence on the limited numbers present. In 1943 the peak catch for the original traps, although late in the season, was actually higher than the 1942 peak, and this in spite of the competition from all the additional traps which must have been great in such a small environment. There can be no doubt that although the 1943 rise in fly population was of late occurrence it was a real and considerable increase and that in that year there were actually many more flies present in the groves than in the previous year. Again, compare the periods August-October in the two years. The numbers of flies caught by 37 traps were fully three times as great as the catches by 14 traps the year before; i.e., in 1943 there was a very high rate of wet-season immigration. In spite of this the incipient rise at the end of 1943 was abruptly checked, and for the first six months of 1944 the total catch for 30 traps was 40 per cent. lower than the total for only 14 traps for the same period of 1942, so that when these 1944 catches are averaged to 14 traps, the characteristic seasonal rise in numbers seems to be altogether missing. It is only on the intrusion of migratory flies from outside sources that there is any sign of an increasing population.

The most striking feature of the figure is the extremely sharp descent in the numbers of flies caught in June, July and August, 1943, after the peak catch for May, despite the continued addition of traps. There can be no doubt that the traps were in fact substantially reducing the population many of which were breeding females and this must have made a critical difference to the potential populations of the groves. The augmentation of the fly population by migration in the latter part of the year was a phenomenon that the traps could not have prevented, but the effects of this immigration must also have been minimised.

An estimate in numerical terms of the amount of reduction brought about in this experiment is a difficult matter. One method of approach is to compare the total catches of flies for complete 12-monthly cycles. Table III summarises the catches illustrated in the figure and shows the numbers of *G. palpalis* and *G. tachinoides* caught in each grove during three successive years.

TABLE III.

Summary of the Yahtori and Zambor Groves Trapping Data from which Figure 5 was drawn.

	Yahtori			Zambor		
	palpalis	*tachinoides*	Total	*palpalis*	*tachinoides*	Total
Nov. 41–Oct. 42, 7 traps in each grove all the time	159	401	560	132	333	465
Nov. 42–Oct. 43, 7 traps in each, augmented in 6th month ...	380	674	1,054	316	977	1,293
Nov. 43–Oct. 44, average of 15 traps in each grove	196	248	444	189	330	519

In so far as the number of flies caught is a measure of those present, it is significant that in both groves a relatively greater decrease took place in the numbers of *G. tachinoides* than in those of *G. palpalis*. In the third year the traps numbered more than twice those used in the first year, yet in Zambor the total number of tsetse caught was only one-tenth greater than in the first year, while in Yahtori it was actually less by one-fifth. This leaves no doubt that the 15 traps in each grove during the year November, 1943-October, 1944, were acting against much reduced populations, but the extent of the reduction is not easily determinable. There was a difference of 75 per cent. between the 14-trap average catches for the six months January-June, 1942, and those for the same months of 1944, and since this covers a period least affected by migrant flies one might be tempted to take the figure as representing the reduction; but such a comparison between different years and with different numbers of traps in use is not valid. Tsetse populations are rarely stable, showing considerable variations from year to year, and there is no doubt that in the early part of 1943 the grove populations were increasing and that they were further augmented by an unusually large migratory influx in the autumn. Short of a catastrophic mortality this would have left the 1944 populations at an appreciably higher level than those of 1942. The catastrophe can be said to have taken place—in the mortality due to the intensified trapping. Whether extermination would eventually have resulted had it been possible to continue the trapping at the highest level is questionable. Under conditions adverse to the tsetse, i.e., with a falling population, the effect of trapping would undoubtedly be greater. In the classic example of Principe every possible means of destruction was used against the fly, and, most important of all, the island represented a truly isolated community cut off by 140 miles of ocean from possible reinfection. But communities of tsetse in favourable circumstances have excellent powers of survival and recovery, and, as in the case of concentrated hand-catching in the same type of environment, eradication, even if possible, could not be permanent. Lloyd (1940) had the same experience in attempting to trap out *G. palpalis* in Maboko Island, Lake Victoria. As long as large numbers of traps were in operation they brought about considerable reduction in tsetse incidence but the flies were never completely eradicated.

(*b*) *Local Control of Extensive Tsetse Populations.*

In the discussion of "siting," examples are given of trapping in extensive and heavily infested flybelt where group of traps after a month's operation showed reductions of 30 - 70 per cent. in the numbers of flies caught. In the Belgian Congo, Lewillon (1945) had the same experience of diminishing catches, but in a more marked degree. The difference between such reductions and those finally attained in the groves must be emphasised. In the groves there were many traps distributed over small areas and dealing with limited fly communities. As there is no evidence to suggest that flies can learn to avoid traps, the eventual fall in the trap catches must have been due to diminishing populations. In the Volta-side experiments, on the other hand, each group of 4 traps was in contact with only a small section of a very high tsetse population in a habitat virtually extending along several hundred miles of river; the surprising thing is that such marked reductions were shown. An explanation can be arrived at from a study of the following figures which illustrate the difference between catches made in thick unbroken fly-belt and those made by a similar set of traps in an open feeding ground in the same fly-belt :—

	Breeding ground in closed fly-belt		Open feeding ground at canoe crossing	
	palpalis	*tachinoides*	*palpalis*	*tachinoides*
Four weeks, Nov./Dec.	99	1,086	53	831
Four weeks, Dec./Jan.	56	547	5	275
Four weeks, Jan./Feb.	38	524	9	311
Four weeks, Feb./Mar.	42	540	6	495

In both cases the initial drop represents a thinning of the population within the range of attraction of the traps. In the dense fly-belt site the subsequent uniform rate of catching can be taken to indicate that the reduction by trapping is being offset by emergence from adjacent breeding grounds and by movement in general. Since the range of attraction of a trap is quite limited, this lowered tsetse incidence can exist in only a small section of fly-belt and is not a real phenomenon of the riverside. In the case of the canoe-crossing feeding ground a higher proportion of the flies present are hungry and are therefore more liable to be trapped; and this might well explain the greater initial reduction. There is a constant flow of hungry flies into these feeding grounds where hosts are always available and this is likely to increase as the dry season advances and the need for more frequent feeds arises. This would explain the subsequent rise in the numbers of flies caught. As before, the capture of a few hundred or even several thousand flies can have no influence on the magnitude of the tsetse population of the fly-belt as a whole, but the removal of those particular flies, which have been in contact with humans, may well be of significance in the control of trypanosomiasis.

(*c*) *Possibilities of Controlling the Disease.*

The results of trapping are so much influenced by extrinsic conditions that this measure cannot compare in efficiency with control by selective clearing on a wide scale, the effects of which can over-ride general as well as local factors. Trapping must remain a subsidiary means of tsetse control to be applied when and where the conditions are favourable. Since eradication of the tsetse by trapping is obviously not a practicable undertaking, even under the most favourable conditions, there remains for consideration the possibility of bringing about control of sleeping sickness by reducing tsetse incidence below the level at which it is a danger to public health or by destroying those flies that are in regular contact with human beings. Factual evidence here is so meagre that the discussion must be largely theoretical.

From the information at present available there appears to be no simple positive correlation between the numbers of tsetse present in a locality and the rate of infection with sleeping sickness that is liable to develop among the inhabitants. In the endemic areas covered by these surveys, communities living close to heavily infested fly-belt have frequently been found with low rates of infection. On the other hand quite high rates of infection can develop in villages, far away from thick fly-belt, where the only contact with tsetse is in very lightly infested groves. It is obvious that the degree of intimacy existing between tsetse and man is of more importance than mere numbers of flies. This fly-man contact is determined by various factors, those that influence the fly, such as the nature and extent of the fly-belt, and those that influence the people, their density, their method of farming, the sources and availability of water; the two sets of factors being at least partly interdependent. For any given complex of factors there is likely to be a certain rate of transmission of trypanosomiasis. A change in any one of those factors would give rise to a change in the transmission rate and in particular it is reasonable to suppose that a general reduction in the tsetse population would, other factors remaining unchanged, lead to a lowered transmission.

The circumstances under which the modification of a tsetse population might be brought about by the use of traps are very limited. Where fly-belt is dense, extensive and heavily infested, trapping is bound to be a waste of time unless it can be carried out so intensively that the cost is likely to outweigh the results. But in areas where the human population is high and the land intensively farmed, the demand for firewood and building poles brings about a natural thinning of the riverine vegetation so that the main concentrations and breeding places of tsetse are found in the sacred groves where the vegetation is untouched. Experiment has shown that in spite of the effects of immigration it is possible to bring about a substantial reduction in the fly-population of a grove. If, then, traps could be placed in all groves along every watercourse in a region of this type it would be expected that the reduction in population would be even greater and certainly more permanent that that demonstrated experimentally, because of the greatly reduced possibilities of migration. Groves, particularly in the dry season, can be sites of a very high degree of man-fly contact. A small fly population, having little inducement to migrate, is in contact with the same group of humans day after day, and it is not surprising that high rates of infection with trypanosomiasis are often associated with villages in such areas. It is reasonable to assume that the rate of infection is in direct proportion to the number of infective bites. A general reduction of the fly population of the groves will cause a proportionate reduction in the number of infective flies and therefore, one may suppose, a corresponding drop in the rate of appearance of new cases of trypanosomiasis. So long as even a few flies remain in contact with infected people the effect of reduced fly populations cannot be more than a partial control of the disease, but it can be achieved at relatively little effort and cost; and the method might conveniently be used temporarily in cases where the local fetish priests need prolonged persuasion before they will give permission for selective clearing in their shrines. The eradication of flies by clearing is infinitely better for the people than the mere reduction of the numbers of flies but it is not more important than the goodwill and the feelings of the people themselves. Their co-operation is essential and will always be well worth the time and tact required to obtain it, particularly if in the meantime the use of efficient traps is helping to check the spread of the disease.

The alternative to causing a general reduction of a tsetse population by trapping is to concentrate on the continuous catching of feeding flies at the points where the majority of trypanosomiasis infections are being incurred. The outcome is likely to be the same, i.e., an incomplete control of the disease, but whereas the former measure can achieve results only in restricted fly-belt, the latter, if successful at all, will be so wherever man and fly are in contact. Indeed, man-fly contact is the key-note in this type of protection; and for the measure to be effective it is essential that there should be a complete understanding of all the relevant factors, in particular of the habits of the people that bring them regularly anywhere in the vicinity of fly-belt or hungry flies. Based on such knowledge the skilful placing of an adequate number of traps is bound, theoretically, to have an effect in reducing the number of infections incurred. The tsetse is an extremely active and cautious creature. The persons on whom it is attempting to feed are also active and are resentful of its attentions. Consequently, flies are apt to be driven from one individual to another and a hungry fly may actually prick four or five people before getting a full meal—a sequence of events which may help the trap because if a fly eventually settles on one it is at least left in peace, and its undisturbed exploring may lead to its capture. These considerations have been borne out by the experiments in siting which showed that the presence of plenty of people increased both the actual numbers of flies caught and the proportion of hungry flies among those captured, or, in other words, that traps are most efficient in places where there is greatest contact between flies and hosts, the very places where they are most needed. If the fly that is about to bite or the fly that has just fed on an infective person is captured, then more has been achieved in preventing the spread of the disease than would result from the destruction of a hundred flies in their fly-belt haunts. The daily catch of flies by a group of traps at a canoe-crossing is an infinitesimal fraction of the whole fly-belt population, but of the flies that come to that particular crossing on any one day in order to bite humans it represents an appreciable fraction.

Another factor that would enhance the protective value of traps lies in their attaining maximum efficiency during the dry season. At this time traps in all situations are catching more successfully than boys and they have even shown the presence of tsetse which routine flyboy catching has failed to detect. During the dry season in northern savanna country the activities of the people are concentrated around available supplies of water which, with the complete drying of small watercourses, and the restriction of surface water to isolated pools even in quite large rivers, are extremely limited. These pools and the deeply dug waterholes often lie within the dry-season habitat of *G. palpalis* and *G. tachinoides* which naturally tend to concentrate around the actual watering places, thus bringing about an even more intimate man-fly contact than is usual during the wet season. For at that time the fly is more dispersed and water is sufficiently abundant for many fly-belt waterholes to be forsaken in favour of open pools closer to the villages. It is reasonable to suppose, though at the moment there is no evidence to support the view, that there is a greater transmission of infection going on during the dry season than during the wet. Thus, the time when the traps are most needed is also the time when they are catching at their best.

(*d*) *Practical Applications.*

The potential value of tsetse traps for the control of sleeping sickness may be summarised as follows :—

Concentrations of traps fail to eradicate tsetse populations even after prolonged operation.

A concentration of well-sited traps, at about 20 to the acre, can appreciably reduce tsetse populations in fly-belt of limited extent and can keep the numbers at a low level. This should bring about a lowering of the incidence of sleeping sickness if the measure is applied on a wide enough scale in a region where the fly-belt is neither heavy nor continuous.

Small groups of traps well placed in feeding grounds bring about a considerable reduction in the number of active, hungry flies in their immediate vicinity but make little difference to the total fly population. This reduction of fly incidence at the points of greatest man-fly contact, even if it be temporary and local, has, in theory, a definite protective value to the individual. The value will be enhanced in inland savanna country by two factors : (*a*) the tendency for people and tsetse to concentrate around waterholes during the dry season, the time when traps are at their highest efficiency ; (*b*) the increased catching power of traps when they are in the closest proximity to humans and animals.

Applying these findings to the problems of control in the field it can be said :—

It would be extremely uneconomic and probably useless to attempt the eradication of tsetse by traps in their present state of development. It is probable that, even with much greater perfection, traps could effect complete control only in very limited environments such as groves. But in such fly-belt, eradication is possible by clearing (at extremely low cost), the results of which are immediate and are not liable to vitiation by the reintroduction of tsetse from outside.

A widespread application of the measures employed is essential if any appreciable and stable control of sleeping sickness by the lowering of tsetse incidence is to be effected (Morris, 1946) ; otherwise control will be local and temporary, and protection of the individual and not general prophylaxis will be achieved. In an outbreak of any magnitude this undertaking would involve enormous numbers of traps, rendering it costly in materials and skilled supervision ; its effects would be liable to reversal with any slackening of effort, and its applicability is limited to localised fly-belts.

Protection by trapping seems to be the line most worthy of attention. It is applicable anywhere, wherever the transmission of trypanosomiasis is found to be taking place. It can be achieved by small numbers of traps at each place so that a reasonable area might be covered at an economic cost. In this way and with persistent effort the cumulative effect might result in a general lowering of the amount of the disease, especially in areas of light infection.

From this point of view traps could be a useful adjunct to clearings. In the case of protective clearings, which are invariably entered by a certain number of flies, a few traps placed round the water-hole or road-crossing might reduce the number of these intruders. In the case of eradicative clearings small groups of traps could be maintained at the limits of the clearings to check invasion from the uncleared fly-belt. In areas of scattered, light infections or in sporadic outbreaks, if the loci of fly infection could be precisely determined, trapping at these points might be sufficient to deal with the situation. But the greatest possibilities lie in places where control by clearing is difficult or impossible. This suggests forest conditions where clearing is unlikely to provide the main solution and will never be easy or cheap. Moreover within the forest, sleeping sickness rarely attains high rates of infection, the exceptions occurring at places where concentrations of people are found, e.g., in towns and at road junctions and ferries. The more usual distribution of the disease in the forest is only in occasional villages and at low rates of infection ; in these villages a high degree of control might be attained by a combination of trapping with other measures such as mass treatment of the people.

The effectiveness of the new insecticides, DDT and " Gammexane ", against *Glossina* introduces a new possibility of control in which contact between the chemical and the insect is established by attracting the latter to bait objects. Experiments in Lawra have shown that with hessian, impregnated with kerosene solutions of DDT, which had been exposed to the weather for five months, contacts of 15 seconds were still fatal to the flies. Traps, the hessian covering of which was impregnated with DDT solution, were just as efficient as ordinary traps, showing that there is no repellent action by the insecticide. Since many flies alight on and explore the traps without entering and being caught, traps treated with DDT must be more effective in destroying tsetse than untreated traps. How this could be proved directly is difficult to envisage ; but it is probably significant that, in a grove where impregnated screens were in use, a considerable mortality was observed among flies caught and retained for liberation at the end of the day by a team of boys making test catches. Normally, in this catch-and-let-go method of sampling fly populations, a very low mortality is experienced. Where traps are being used for protective purposes the additional cost of treating the covering material with DDT at intervals of two or three months would be very small. Indeed, the catching cage might be dispensed with provided the concentration of insecticide on the fabric was maintained, and this would simplify construction and greatly reduce costs. But it would be advisable to retain one or two complete traps in every group of bait objects to ensure that good sites were chosen and to give a measure of the attendances of the tsetse.

During their discussions of these trapping experiments and their attempts to find explanations of the various results, the writers have become more and more aware of the inadequacy of the conception of a tsetse as a mere bunch of tropisms reacting automatically to each set of stimuli as it is encountered. The tsetse fly is one of the most specialised members of a highly developed family of insects. The need of the adult fly, throughout a comparatively long life, to make frequent and regular contacts with special food hosts and at the same time to avoid the dangers arising from those hosts, from its predators and from the harsh environment to which it is frequently exposed, has led to its developing a complex and versatile behaviour. Success in trapping this fly depends on a knowledge of these complex habits. The inadequacy of the present knowledge has been brought home to the writers during these investigations. Further understanding should make possible the development of traps and trapping into far more successful, and perhaps valuable, weapons in the armoury against the tsetse.

A final point, that is so frequently overlooked, is worth mentioning. Undoubtedly the drive and ingenuity displayed in trapping can be greatly stimulated by treating tsetse as trout and partridges, and measuring success by the size of the bag, but the size of the bag may become an end in itself. It should be borne in mind that careful relation of trapping operations to the various problems of trypanosomiasis can be of greater value to the community than the mere destruction of large numbers of flies.

Summary.

From 1940 up to the time of writing, experiments have been carried out in the savanna country in the north of the Gold Coast on the trapping of *Glossina palpalis* and *G. tachinoides*, which are the most important vectors of sleeping sickness in West Africa.

The habitat of these tsetse is described, with special reference to their feeding grounds, since those are the places where trapping can be most effective and control of the flies is most desirable.

A new type of trap, the Animal trap, was designed to meet the feeding habits of the two species of tsetse whose main hosts in the country where sleeping sickness is prevalent are man and domestic animals.

The construction and maintenance of the Animal trap are described, the essential feature of which is a barrel-shaped body standing out well under good lighting and easily visible from all angles.

Traps with a standardised 2-feet-long body raised 1 foot from the ground combined optimum catching with convenience in construction and portability.

Light brown or khaki covering was slightly better than black, and rough material was decidedly better than smooth. Under poor lighting conditions these differences were less marked.

Comparisons over long periods showed that the Animal trap caught over 20 times as many flies as the Harris trap which was not at all suitable for *G. palpalis*;

about 10 times as many flies as Swynnerton's A.S.B. trap; and about seven times as many flies as Chorley's Crinoline trap. 21 202

The correct siting of traps is of the greatest importance.

Traps catch well anywhere in fly-belt but are most effective in open feeding grounds such as ferries, fords, waterholes and animals' drinking places.

The regular presence of an abundance of hosts is the most important single factor for good siting, and the nearer the traps can be placed to natural hosts the more effective they will prove.

Traps should be placed if possible with their axes at right angles to the regular lines of movement of the flies.

Good visibility of the trap from as wide an angle as possible is of great importance. Traps about five yards from the edge of the fly-belt catch twice as well as traps right at the margin of the bushes and considerably better than traps 40-50 yards out.

Good lighting, especially from overhead, is an advantage but by no means essential.

Good sites remain consistently good as long as the environment is unchanged, and bad sites remain bad.

There is a tendency for the numbers of flies caught to fall by as much as 70 per cent. during the first few weeks when traps are placed in undisturbed fly-belt. This is the effect of skimming off a part of the fly population in the immediate vicinity of the traps and does not represent a real reduction in population.

Comparison of tsetse catches by groups of five traps operating continuously, and by flyboys at the rate of 20 flyboy-days a month from parallel series of observations, gave the following facts for which explanations are offered.

Traps relative to flyboys are more efficient in taking *G. tachinoides* and less efficient in the cases of *G. palpalis* and *G. morsitans*. Flyboys, however, catch a higher proportion of female *G. tachinoides* but a lower proportion of females of the other two species.

It is considered that Animal traps represent the regular hosts of *G. tachinoides* and so attract non-feeding males as well as hungry flies of both sexes, whereas in the cases of *G. palpalis* and *G. morsitans* the traps represent something outside the range of their preferred hosts and so attract only hungry flies bent on obtaining a meal.

Traps appear to have a greater efficiency relative to flyboys in groves and clearings where the numbers of flies are small, than in sites in or near heavy and extensive fly-belt with high fly populations. This is possibly the result of competition which would be most evident in small tsetse populations and which would act in favour of the continuously present traps.

There is a striking difference in the annual rhythms of catches by flyboys and by traps; the former showing a double-peaked curve with maxima at the beginning and in the middle of the wet season, the latter with a single peak towards the end of the dry season and a minimum during the rains.

The efficiency of traps relative to flyboys is greatest in January and least in September. In an estimate based on all the available data and comparing 150 trap-days with 20 flyboy-days monthly, the traps at their best were 10 times as good as the flyboys, and at their other extreme were as low as one-fourth of the flyboys' catches.

It is considered that this maximum efficiency in the dry season is not due to the flies' attraction to shade but that it is a complex result of the circumstances which bring about optimum conditions for trapping in the dry season, when the weather frequently puts flyboys at a disadvantage, and the most adverse conditions for trapping in the wet season when they enjoy certain advantages of mobility.

The seasonal rhythm of trap catches has demonstrated the presence of a considerably greater fly population in the height of the dry season than is suggested by the magnitude of boys' catches.

Three possibilities of trapping in the control of sleeping sickness are examined: (1) for extermination of the tsetse; (2) for reduction of the fly population; (3) for destruction of feeding flies in contact with people.

Regarding the first possibility it had been found that continuous catching by teams of four flyboys could eliminate *G. palpalis* and *G. tachinoides* from small groves after 8–12 weeks' continuous catching but that, since none of these groves was completely isolated, repopulation took place by immigration, especially in the wet season. Small numbers of traps, 5–7 to the acre, made no apparent difference to limited tsetse populations but concentrations of traps up to 20 to the acre brought about considerable reductions and checked the effects of immigration, but with no approach to extermination of the flies.

Eradication of the tsetse by traps is impossible but reduction of fly populations, if it can be effected over the main area of an epidemic, should, in theory, result in a partial control of trypanosomiasis.

Small groups of traps well sited in feeding grounds bring about considerable reductions in the numbers of active, hungry flies in contact with their hosts although hardly affecting the main fly community. This, in theory, will have a definite protective value to the community, enhanced by the fact that traps show increased catching powers when in close proximity to man and animals and that there is a tendency for people and tsetse to concentrate around waterholes (thus coming into the most intimate contact), during the dry season, the very time when traps are working at maximum efficiency.

It is in this protective role that traps seem to show their greatest possibilities for use in the control of sleeping sickness. The measure is applicable anywhere. It needs only small numbers of traps at each place where infection is being transmitted so that a reasonably big area could be covered economically. It might be a useful adjunct to clearings which fail to exclude the tsetse sufficiently. Traps so used might prove a valuable supplementary control-medium in places, such as the forest, where clearing is unlikely to be easy or cheap. Finally, experiment has shown that the retention of toxicity to *Glossina* of DDT-impregnated hessian exposed to the weather is sufficient to justify the application of DDT to the material covering the traps in order to increase their killing power.

Acknowledgements.

The industry and ingenuity of our Field Assistant, Josiah Odobill, who constructed the traps and supervised all subsequent routine observations, and the reliability of our Trapboys, Braimah Fulani and Bukari Mossi, who carried out the visits and repairs under all conditions of weather, deserve the highest praise. It is due to their steady work, and to that of our Flyboys and Recorders, that such a mass of data could be collected.

References.

CHORLEY, C. W. (1933). Traps for tsetse flies of the "crinoline" and "ventilator" forms.—Bull. ent. Res., **24**, pp. 315-318.

DA COSTA, B. F. B., SANT' ANNA, J. F., DOS SANTOS, A. C. & DE ARAUJO ALVARES, M. G. (19~~4~~6). Sleeping sickness: a record of four years' war against it /1 in the Island of Principe. Trans. by J. A. Wyllie.—261 pp. London, Baillière, Tindall & Cox.

19 78

HARRIS, R. H. T. P. (1930). Report on the trapping of tsetse flies.—8 pp. Pietermaritzburg, Natal Witness.

——. (1938). The control and possible extermination of the tsetse by trapping.—Acta Conv. ter. trop. Malar. Morb., Amsterdam, **1**, pp. 663-677.

HENRARD, C. (1934). Quelques essais de capture de *Glossina palpalis* au moyen de divers types de piège Harris du Stanley Pool.—Ann. Soc. belge Méd. trop., **14**, pp. 263-276.

JACK, R. W. (1939). Studies in the physiology and behaviour of *Glossina morsitans*, Westw.—Mem. Dep. Agric. S. Rhod., no. 1, 203 pp.

——. (1944). The life economy of a tsetse fly.—Rhod. agric. J., **41**, pp. 25-38.

LEWILLON, R. (1945). Le piégeage expérimental de *Glossina palpalis* à la Mission Médical du Kwango.—Rev. Trav. Sci. méd. Congo Belge, **4**, pp. 45-57.

LLOYD, H. M. (1940). Trapping experiments in Maboko Island.—Tsetse Res. Rep. Tanganyika, 1935-38, pp. 42-44.

MALDONADO. (1910). Bull. Sleep. Sickn. Bur., **2**, p. 26.

MORRIS, K. R. S. (1946). The control of trypanosomiasis by entomological means.—Bull. ent. Res., **37**, pp. 201-250.

SWYNNERTON, C. F. M. (1933). Some traps for tsetse flies.—Bull. ent. Res., **24**, pp. 69-105.

——. (1936). The tsetse flies in East Africa.—Trans. R. ent. Soc. Lond., **84**, pp. 1-579.

SYMES, C. B. (1935). Outline of work on *G. palpalis* in Kenya.—E. Afr. med. J., **12**, pp. 263-281.

ZUMPT, F. (1936). Die Bekämpfung der Tsetsen mittels Fallen im Pflanzungsgebiet des Kamerunberges.—Arb. physiol. angew. Ent. Berl., **3**, pp. 197-202.

——. (1937). Untersuchungen über Tsetsefliegen und deren Bekämpfung im Pflanzungsgebiet des Kamerunberges.—Tropenpflanzer, **40**, pp. 1-31, 65-76.

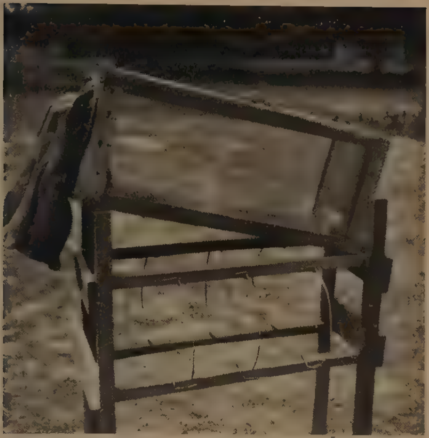


FIG. 1. Skeleton of Animal trap, showing simple method of slipping cage into frame.

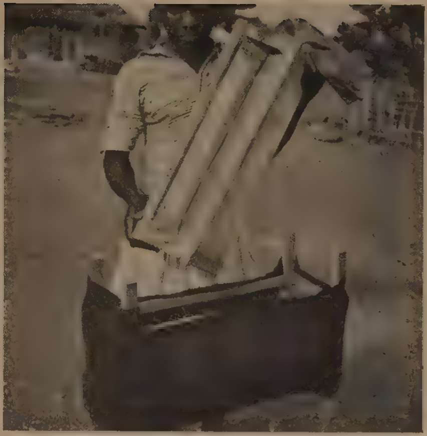


FIG. 2. Cloth-covered Animal trap with cage removed to show method of tying entrance slit to prevent gaping or closing.

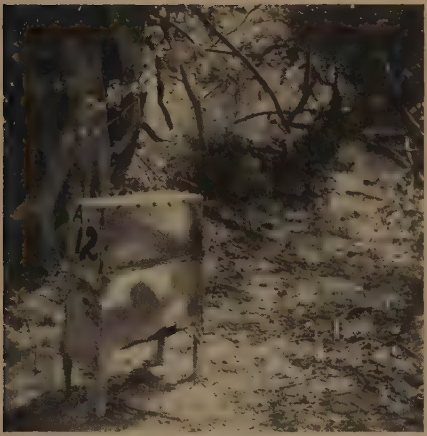


FIG. 3. Animal trap covered with goat skin set on a path through fly-belt.

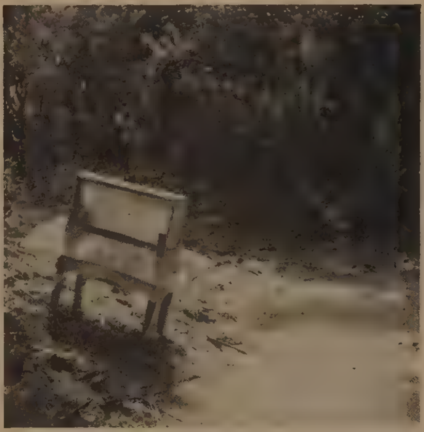


FIG. 4. The Awning modification of the Animal trap, showing how the natural form of the trap's body is masked.

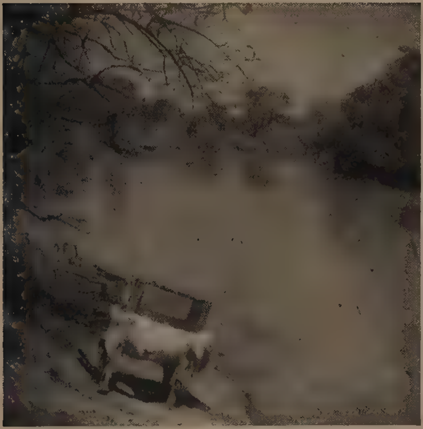


FIG. 1. Sheep skin covered trap set on a promontory opposite heavy fly-belt.

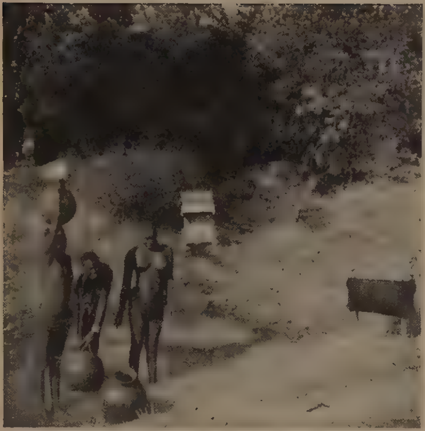


FIG. 2. Zambor Grove, with an ASB trap in the best site, on the direct route followed by tsetse between fly-belt and water hole.

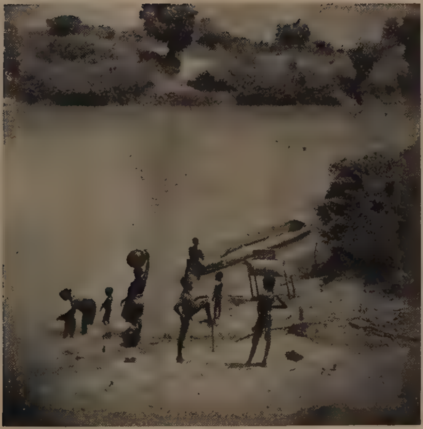


FIG. 3. A tsetse feeding ground on the Black Volta near Lawra, where the regular presence of hosts was conducive to high catches. A large-sized Animal trap on the right stands between fly-belt and hosts.

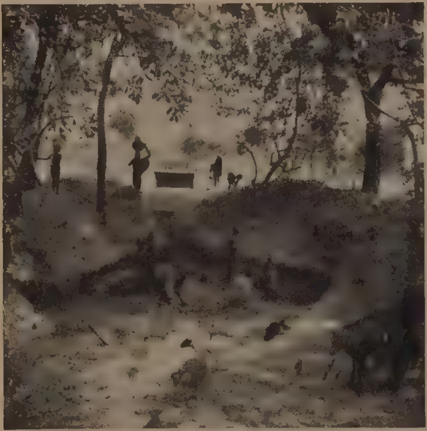


FIG. 4. A sacred grove (from the inside) where the daily concentration of men and animals in intimate contact with tsetse affords maximum opportunities for transmission of trypanosomiasis. In such places trapping might be a valuable control means.

GLOSSINA TACHINOIDES IN NORTH-EAST AFRICA.

By D. J. Lewis.
Medical Entomologist, Sudan Medical Service.

Glossina tachinoides, Westwood, 1850, is a common tsetse fly and an important vector of trypanosomiasis in parts of West Africa.

It was reported from Arabia by Carter (1906), and Austen (1911) naturally believed that it occurred across Africa from east to west. Later surveys, however, did not fill in the gap, and Buxton (1939), reviewing a paper on Diptera of Ethiopia and Italian Somaliland, wrote: " The paper gives no information on one most curious riddle. *G. tachinoides* is a West African species, its eastern limit being the Shari River about 18°E. But it also occurs in the hills behind Aden, the gap in its range being about 27°, say 1,600 miles. The present paper contains no record of *tachinoides* (which one might expect to be found in some part of the area under review) ".

Ghidini (1939) reported *G. tachinoides* from the Gambeila district of western Ethiopia. In March, 1948, Dr. J. F. E. Bloss, of the Sudan Medical Service, found a single female tsetse fly at Kigille (8°, 42′ N.; 34°, 04′ E.) in the Anglo-Egyptian Sudan, which he recognised as an unusual species and sent to the writer. It proved to be *G. tachinoides*. Kigille is near the border of the Gambeila district, in a remote area, described by Corfield (1938), which is difficult to reach at most times of year. It is a small police post on a stream flowing from the Ethiopian hills towards the swamps of the Nile Valley. The fly was taken when biting the collector's arm near a pool in the bed of the stream.

G. tachinoides has not been found anywhere else in the Anglo-Egyptian Sudan although many collections of *G. palpalis* subsp. *fuscipes* and *G. morsitans* have been made in the last 45 years, including those by Bloss (1945) and the present writer. There still appears to be a wide gap in the distribution of *G. tachinoides*.

The gap is so remarkable that the original record from Arabia has sometimes been questioned, in spite of Carter's detailed account; Patton and Evans referred to it as a very doubtful record lacking confirmation and omitted it from their map, and Smart (1948) described it as a dubious record to be accepted with caution. Although the gap is much less than 1,600 miles it is nevertheless considerable. In the Sudan it includes the great swamps of the Sadd region, where conditions are unsuitable for tsetse flies, and a large area to the west through which flow many tsetse-infested tributaries of the Nile system. It is not known why *G. tachinoides* is absent from these tributaries, but it is interesting to note that many insects in the Sudan show great discontinuity in their distribution, which appears to have been caused by past changes in climate.

Scott's (1939) unsuccessful search for *G. tachinoides* in one of the original localities in Arabia perhaps suggests that its foothold is somewhat insecure in that country.

I am much indebted to Dr. F. van Emden, of the Commonwealth Institute of Entomology, for confirming the identification of the Kigille specimen which has been placed in the British Museum.

References.

Austen, E. E. (1911). A handbook of the tsetse flies.—London, Brit. Mus. (Nat. Hist.).

BLOSS, J. F. E. (1945). The Meridi outbreak of sleeping sickness.—Trans. R. Soc. trop. Med. Hyg., **36**, pp. 59-76.

BUXTON, P. A. (1939). [Review.]—Trop. Dis. Bull., **36**, p. 214.

CARTER, R. M. (1906). Tsetse fly in Arabia.—Brit. med. J., **1906**, 2, p. 1393.

CORFIELD, F. D. (1938). The Koma.—Sudan Notes, **21**, pp. 123-165.

GHIDINI, G. M. (1939). Nuovi dati sulla distribuzione delle glossine nelle terre dell'Impero.—Riv. Biol. colon., **2**, pp. 329-333.

PATTON, W. S. & EVANS, A. M. (1929). Insects, ticks, mites and venomous animals of medical and veterinary importance. Part I.—Medical. Liverpool Sch. trop. Med.

SCOTT, H. (1939). A journey to the Yemen.—Geogr. J., **93**, pp. 97-125.

SMART, J. (1948). A handbook for the identification of insects of medical importance.—2nd. ed. London, Brit. Mus. (Nat. Hist.).

WESTWOOD, J. O. (1850). Observations on the destructive species of Dipterous insects—Proc. zool. Soc. Lond., pt. 48, pp. 258-270.

ON THE INDIAN SPECIES OF *RODOLIA* MULSANT (COLEOPTERA—COCCINELLIDAE).

By A. P. Kapur, Ph.D.
Commonwealth Institute of Entomology.

E.H.N.

Species of *Rodolia* Mulsant (1850) are important as predators of mealybugs, some of which are notorious for their injury to plants. *Rodolia cardinalis* Mulsant is a classical example of a species that has been successfully introduced into several countries against the cottony-cushion scale, *Icerya purchasi* Maskell. A campaign against the latter, in South India, has been in progress for some time, and a number of local Coccinellid predators, collected by the staff responsible for the work, were sent for identification to the Commonwealth Institute of Entomology. The present study is based mainly on this material, and also on that in the British Museum (Natural History). All the known Indian species are redescribed and three new ones added to the list.

I am grateful to Dr. O. W. Richards and Dr. T. H. C. Taylor for their encouragement in the preparation of this paper.

Rodolia Mulsant, 1850, p. 902.

Genotype : *Rodolia ruficollis* Mulsant.

Crotch (1874) was the first to fix *R. ruficollis* Mulsant as the type of the genus. Owing apparently to an error in the posthumous printing of his work, *ruficollis* appeared on the next page under the genus *Vedalia* Mulsant (and conversely, the genotype of the latter under *Rodolia*). This confusion seems to have led Gorham (1901) to propose *Rodolia rubea* Mulsant as the type. The opinion is held here that *ruficollis,* which is one of the species included in *Rodolia* by Mulsant when he described the genus, remains the type and the fact that it appeared under *Vedalia* in Crotch's monograph should not disqualify it from being regarded as the genotype of *Rodolia.*

A brief description of the genus is as follows :—

Small to moderately large, pubescent Coccinellinae. Body shortly oval to hemispherical; head rather large, transverse; eyes large, finely facetted and pubescent, not emarginate or notched on the inner margin; epistoma weakly emarginate at the anterior border, and obliquely notched laterally to expose the bases of antennae which are short, nearly as long as the distance between the two eyes, eight segmented, the first two segments being large and oval, the next three short and slender, and the apical three forming a more or less fusiform club; labrum well-developed, transverse, convex and sub-rounded on the anterior and lateral margins. Pronotum nearly twice as broad as long, convex, deeply emarginate anteriorly, anterior angles broad or rather narrowly rounded, lateral margins rounded or nearly straight, basal margin devoid of any border, rounded except for a short truncate part in the middle opposite the scutellum; elytra wider than pronotum, moderately to strongly convex, rounded at the shoulders, very narrowly margined along the external border; epipleurae wide, especially in the basal half, concave, inclined and devoid of foveae; prosternum nearly as wide as the pair of coxae which almost conceal it except for a very narrow anterior margin and the medium part which is more or less sharply raised anteriorly, inclined posteriorly and variously shaped in different species; abdomen with six visible sternites; abdominal lines complete, bow-shaped and hardly extending to the middle of the segment; legs sufficiently robust, nearly compressed, femora sulcate or deeply furrowed distally, tibiae obliquely furrowed and angular at the middle of the external margin; tarsal claws bifid with the inner division shorter than the outer.

The chief features which distinguish the genus from other closely related genera of the tribe are: the absence of any emargination of the eyes, the narrow and weakly developed prosternum and the median part of which is raised anteriorly, and the furrowed tibiae with the external margin angled.

Excepting the shape, colouration and size, there are few reliable external characters by which closely related species may be distinguished from one another. Generally speaking the pubescence is moderately long and dense and the punctation fine and close and a little finer on the pronotum than on elytra. However, the genitalia in both sexes offer excellent diagnostic characters.

KEY TO THE SPECIES.

1. Elytra reddish brown to carmine-red, with black spots..........................2

Elytra usually uniformly yellowish brown, brown, reddish or carmine-red, rarely fuscous, sometimes fuscous only along the margins or at the apices..4

2. Body sub-hemispherical, 3·25 mm. long, elytron with one small black spot situated a little anterior to the middle and as distant from the suture as its own diameter (South India)..*netara*, **sp.n.**

Body obtusely oval or sub-ovate, with more than one spot on elytron.........3

3. Body obtusely oval, usually 3·8-4·3 mm. long; head black; pronotum with a black band at the base; elytra with black sutural and apical borders, and each with two black spots: one curved round the inner side of the shoulder-boil, and the other discal, at 3/5ths the length of elytron and appearing as if composed of two fused spots (widely distributed over the world; in India mainly in the South)..................................*cardinalis* (Mulsant)

Body sub-ovate; usually 4·8-5·0 mm. long; head not black; pronotum with a small transverse band (as if formed of two fused spots) in the middle of the base; elytron with three spots: two (roundish) placed transversely a little before the middle and one (oval) in the apical third of elytron (North India)...*guerini* (Crotch)

4. Body shortly oval and slightly narrowed posteriorly, 5-5·6 mm. long; elytra entirely or partly testaceous, reddish testaceous, nearly red or light piceous, sometimes only the margins or part of them fuscous; punctation fine and close; median part of prosternum narrowed and less conspicuously raised anteriorly (widely distributed)..*fumida* Mulsant

Body sub-hemispherical, not narrowed posteriorly, median part of prosternum more distinctly raised...5

5. Body small, nearly 2·3 mm. long; head, pronotum and greater part of elytra reddish testaceous to carmine-red, the apical third and lateral border of elytra dark brown (South India)......................................*minima*, **sp.n.**

Body medium to large, over 3·0 mm. long..6

6. Body nearly 6 mm. long, head and pronotum orange-yellow, elytra reddish brown, with the external border orange (North India)...*ruficollis* Mulsant

Body less than 5·5 mm. long, head, pronotum and elytra uniformly coloured...7

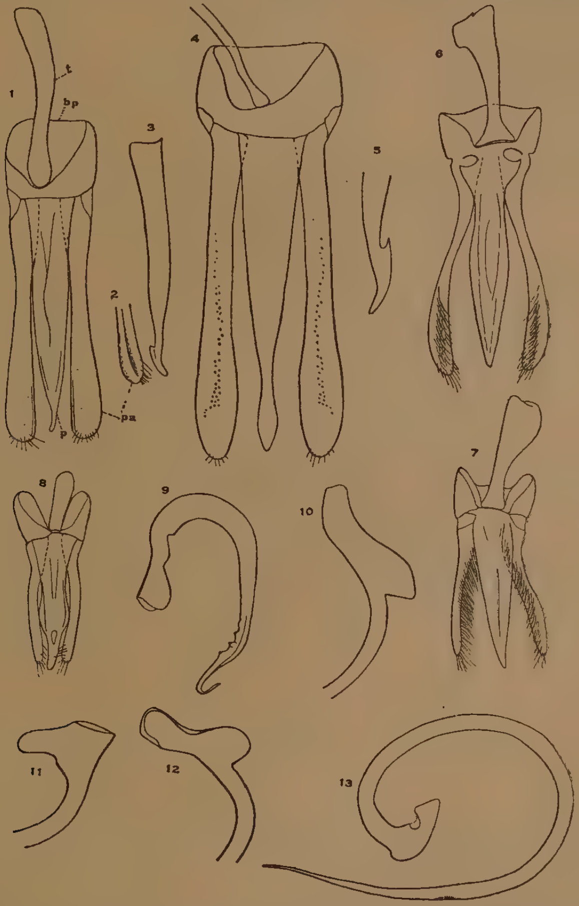
7. Body 4·8-5·2 mm. long, rusty, opaque and matt, finely and closely punctate and with long, yellowish and less dense pubescence; anterior angles of pronotum broadly rounded (Andaman Islands)...............*andamanica* Weise

Body 3-4·25 mm. long, upper surface shiny, anterior angles of pronotum narrow; pubescence denser and shorter..8

8. Body larger (3-4 mm. long), pubescence greyish or slightly yellowish, and close; elytral punctures obsolete (India and Ceylon)......*breviuscula* Weise.

Body smaller (3 mm. long), reddish or carmine-brown, pubescence yellowish, elytral punctures impressed (Central and South India)......*amabilis*, **sp.n.**

The differences in the structure of the genitalia have not been included in the above key, as these can best be observed and compared with the diagrams given in the paper. *Rodolia cardinalis* (Mulsant) is an introduced species in India and is too well-known to be redescribed. *R. rufopilosa* (Mulsant) has not been included in the key because it probably is not an Indian species, although Gorham (1901)



Figs. 1–9.—Parts of male genitalia. (1) *Rodolia fumida*, t (trab), bp (basal plates), p (penis) pa (paramera); (2) lateral view of apex of parameron; (3) lateral view of penis; (4) *R. andamanica*; (5) lateral view of apex of penis. (6) *R. breviuscula*; (7) *R. amabilis*, sp.n; (8) *R. minima*, sp.n.; (9) sipho.

Figs. 10–12. Siphonal capsules. (10) *R. andamanica*; (11) *R. breviuscula*; (12) *R. fumida*.

Fig. 13. Sipho of *R. amabilis*.

(All magnifications × 33.)

records it from Belgaum (India). This material from Belgaum, in Mr. H. L. Andrewes' collection, now in the British Museum, has been examined, and also several examples of the species from China (including what is believed to be the type) and Penang, as mentioned by Crotch (1874), and it is not considered that the Belgaum material is *rufopilosa.* The most obvious differences are the shortly oval, less convex and smaller body of the Belgaum examples which seem to belong to *R. fumida,* as against the sub-hemispherical, strongly convex and larger body of *R. rufopilosa,* which is 6·1 mm. long.

Rodolia fumida Mulsant (figs. 1-3, 12 and 15).

Rodolia fumida Mulsant, 1850, p. 904.

Rodolia roseipennis Mulsant, 1850, p. 904; Crotch, 1874, p. 281.

Rodolia arethusa Mulsant, 1853, p. 254; Crotch, 1874, p. 281.

Rodolia testicolor Mulsant, 1853, p. 255; Crotch, 1874, p. 281.

Body shortly oval, slightly narrowed posteriorly, moderately convex; coloration very variable, being rose-red, reddish-brown, brown or dark brown. Weise (1892) lists the following varieties which, judging from the extensive material examined, occur with equal frequency in North and South India :—

var. *a* : elytra entirely testaceous, reddish testaceous or rose-red (*roseipennis* Mulsant).

var. *b* : as in var. *a,* but sutural, and basal margins and anterior part of lateral margin dark brown.

var. *c* : as in var. *a,* but elytra dark except in the discal area.

var. *d* : elytra entirely dark brown or piceous.

Dorsal surface with pubescence whitish grey or light yellow; punctation fine, close and shallow, a little finer on the head and pronotum than on the elytra; pronotum with the lateral margins rounded and the anterior angles broadly rounded; shoulder-boil of elytra more or less distinct; median part of prosternum nearly twice as long as broad, gradually narrowing and only slightly raised anteriorly; sixth abdominal sternite shallowly emarginate at the apex, more widely so in male than in female; male genitalia as shown in figs. 1-3 and 12, the shape of the long and spathulate paramera, tapering penis and the bi-lobed siphonal capsule being characteristic; the two parts of ninth sternite of female elongate, gradually narrowing to a rounded apex; spermatheca bulbous with a narrow and short base (fig. 15).

Length 5-5·6 mm., width 4-4·2 mm.

GEOGRAPHICAL DISTRIBUTION :—Type locality: Bengal; also recorded from Madagascar (*Sicard,* 1909). The examples examined are from the following localities :—

INDIA : Assam : Sylhet; Manipur (*Doherty*). Bengal : North Bengal; Darjeeling (*G. Rogers*). United Provinces : Almora (*H. G. Champion*); Ranikhet (*H. G. C.*); Sitapur (*H. G. C.*). Punjab : Murree Hills (*H. Roberts*); Lahore, -.xii.1912 (*B. Das*); Lahore, feeding (?) on Aphids on peach, 4.ii.1936 (*A. P. Kapur*); Delhi, feeding on Aphids and Coccids on cowpea, 4.vii.1938 (*A. P. Kapur*); Delhi, larva and adult feeding on *Icerya* sp., 5.vii.1945 (*M. Bose*). Sind : Karachi (*T. R. Bell*). Nagpur, feeding on giant mealybug, 9.vii.1944 (*V. P. Rao*). Belgaum (*H. L. Andrewes*). Nilgiri Hills (*H. L. Andrewes*).

CEYLON : Balaogoda (1,775), 13-16.iii.1882 (*G. Lewis*); Hambantota, 31.x.1908, 7.xi.1908 (*T. B. Fletcher*).

BURMA : Ruby mines (*Doherty*).

Rodolia ruficollis Mulsant, 1850, p. 903 (fig. 14).

Body large, sub-hemispherical; head and pronotum orange-yellow, elytra reddish brown with the external border orange; underside obscurely reddish yellow or brown; pubescence yellowish, moderately long, semi-erect, and dense; punctation fine and close, more impressed on elytra than on pronotum; median part of prosternum elongate, slightly narrowed and raised anteriorly; male genitalia not known; sixth sternite in female entire; the two parts of the ninth gradually narrowing to a sub-rounded apex, spermatheca large and globular distally, with a moderately long trunk which is wider at the base (fig. 14).

Length 5·8-6·1 mm., width 5·3-5·6 mm.

Geographical Distribution :—Type locality : Bengal. The examples examined are from the following localities :—

India : Assam, Patkai Mountains (*Doherty*); Nilgiri Hills (*H. L. Andrewes*).

Rodolia guerini (Crotch).

Vedalia guerini Crotch, 1874, p. 282.

Rodolia immsi Weise, 1912, p. 120.

Rodolia 6-maculata Korschefsky, 1940, p. 2 **(syn. nov.)**.

Body shortly oval, slightly narrow posteriorly; convex; dorsal surface reddish brown to testaceous with a sub-transverse and black or ill-defined, fuscous marking at the middle of the base of pronotum and three black to piceous spots on each elytron, two of these placed transversely a little anterior to the middle of elytron and the third, which is larger, in the centre of the apical half of elytron. Usually the elytral spots are well defined, rounded or slightly oval, sometimes they are indistinct in outline and piceous. Underside slightly paler than the upper and dark brown in parts of metathorax. Pubescence greyish white, fairly long and dense; punctation on the whole distinct, close and fine, more so on the head and pronotum than on elytra where a few slightly coarser punctures are irregularly dispersed (as seen under high magnification). Pronotum with the anterior angles a little narrower than in *fumida*; shoulder-boil of elytron rather distinct; prosternum nearly twice as long as wide, slightly narrowed and raised anteriorly; the sixth abdominal sternite weakly emarginate in male and entire in female. Male genitalia very much like that of *fumida* but with the lateral margins of penis sub-parallel except very near the narrowed apex.

Length 4·8-5·0 mm., width 4·0 mm.

Geographical Distribution :—Type locality : Pondicherry; South India (Dehra Dun, United Provinces, India, for *immsi* and 6-*maculata*). The examples examined are from Dehra Dun (*E. P. Stebbing*) and Haldwani district, United Provinces (*H. G. Champion*).

Remarks. In Korschefsky's (1931) catalogue, *immsi* is regarded as synonymous with *guerini*. The form 6-*maculata* also appears to be the same as *guerini* and the presence of a few slightly coarser elytral punctures in the former is the only important difference that one can note in their descriptions. Such punctures are present in all the specimens labelled *guerini* in the collection studied.

Rodolia andamanica Weise, 1901, p. 93 (figs. 4, 5, 10 and 16).

Body sub-hemispherical, upper and underside uniformly rusty or light testaceous opaque and matt; pubescence yellowish, short and dense; head and pronotum very finely and closely punctate, elytra with similar but slightly coarser punctures; pronotum with the lateral margins and the anterior angles broadly rounded; elytra without a defined shoulder-boil; prosternum narrow, distinctly twice as long as wide, slightly narrowed and raised anteriorly; the sixth abdominal sternite entire in the females and notched in the male. Male genitalia as shown in figs. 4, 5 and 10; penis abruptly narrowed before the apex, paramera broad and spathulate, and sipho broadly curved with the apex drawn out in the form of a long thread; spermatheca in the female as shown in fig. 16.

Length 4·8-5·2 mm., width 4·5-4·8 mm.

Geographical Distribution :—Type locality : Andaman Islands. The examples examined are also from the Andaman Islands (*Roepstorff*).

Rodolia breviuscula Weise, 1892, p. 26 (figs. 6, 11 and 21).

Body hemispherical, testaceous on the upper and under sides; pubescence greyish white or light yellow, semi-erect, moderately long and dense; punctation fine, close and rather shallow, a little finer on the head and pronotum than on elytra; pronotum with the lateral margins only slightly rounded and the anterior angles acute; prosternum nearly as long as broad at the base, slightly narrowed and raised anteriorly; sixth abdominal sternite in male fairly deeply emarginate at the apex, in female entire; male genitalia as shown in figs. 6 and 11; penis slightly narrowed between the base and the middle of its length; ninth sternite in female long and tapering, spermatheca (fig. 21) sausage-like and curved to form three-fourths of a circle.

Length 3·25-4·0 mm., width 2·7-3·3 mm.

Geographical Distribution :—Type locality : Mandar (Chota, Nagpur, Central Provinces, India); also recorded from Ceylon (Korschefsky, 1931). The examples studied are from the following localities :—

India : South India : Shevaroys, Yergaud (4,500 ft.), April-May, 1931 (*Y. Rao*); Udipi, South Kanara, feeding on *Drosichiella* sp., 8.v.1944 (*V. P. Rao*); Sarapanemane, South Kanara, feeding on *Icerya* sp., 10.v.1944 (*V. P. Rao*); Chandramalai Estate, Nelliampathy Hills, feeding on *Drosichiella* sp., on *Citrus* sp., 22.iv.1944 (*V. P. Rao*); Anakapulle, feeding on *Icerya* sp. attacking *Achras sapota*, 16.vi.1944 (*V. C. Upadhyayula*); Shimoga, feeding on *Icerya seychellarum* Westwood, 7.viii.1944 (*Kumar*); Devarshola, feeding on *Icerya aegyptiaca* Douglas, 17.xii.1944 (*V. P. Rao*); Kodanari Estate, Kukul Valley, feeding on *Icerya purchasi* Maskell, 8.vi.1944 (*V. P. Rao*); Chittamannar, 1947; Travandrum, 1947.

Ceylon : Peradeniya, -.i.1910 and -.xii.1918; Ragalla, predacious on *I. purchasi*, 10.xi.1917 (*J. C. Hutson*).

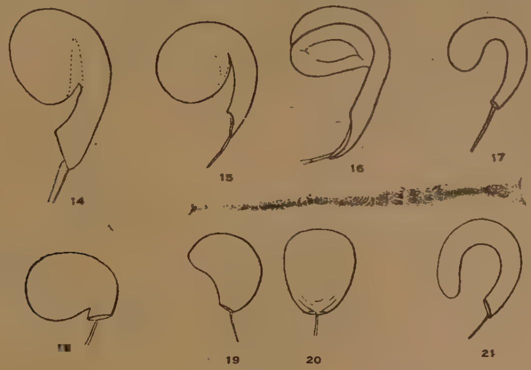
Rodolia amabilis, sp. n. (figs. 7, 13 and 18).

Body sub-hemispherical, usually uniformly reddish brown with a slight tinge of carmine, sometimes brown; pubescence yellowish, short, semi-erect and moderately dense; punctation very fine, close and rather obsolete; pronotum with the lateral margins slightly rounded and the anterior angles narrow; prosternum nearly as long as wide and raised anteriorly; sixth abdominal sternite in male more distinctly emarginate than in female; male genitalia as shown in figs. 7 and 13, the compact row of long hairs on the entire inner margin of paramera being very characteristic; female with the ninth sternite long and tapering to a pointed apex, spermatheca (fig. 18) large, shortly oval, except for a short and narrow basal part.

Length 3·25 mm., width 3·0 mm.

Type in the British Museum; locality : South India, Mysore State, Bhatteshally, feeding on *Icerya purchasi* Maskell, 24.v.1947 (Plant Protection Adviser, India).

Paratypes : 12, in the British Museum, the Indian Museum (Plant Protection Adviser's bequest), and in the author's collection. Of these, five have the same data as the type; the remainder were also feeding on *I. purchasi* and have the following data. South India, Mysore State, Bangalore, 25.xi.1944 (*V. P. Rao*); Thavarekere, 1.xii.1944 (*V. P. Rao*); Talliar, 1.i.1944 (*V. P. Rao*); Periakulam, 19.ix.1944 (*G. S. Chelladorai*).



Figs. 14–19, 21.—Lateral views of spermathecal capsules. (14) *Rodolia ruficollis*; (15) *R. fumida*; (16) *R. andamanica*; (17) *R. netara*, sp. n.; (18) *R. amabilis*, sp. n.; (19) *R. minima*, sp.n.; (21) *R. breviuscula*, sp.n.

Fig. 20.—Front view. *R. minima*, sp. n.

(Free-hand drawings of no fixed magnifications.)

Rodolia netara, sp. n. (fig. 17).

Sub-hemispherical, head carmine-red to dark brown, pronotum and elytra carmine-red, except for a rounded black spot on each elytron. The spot is small, its diameter being about one-fourth the greatest width of elytron, and lies a little anterior to the middle of the suture and nearly as far from the suture as its own diameter; underside carmine-red, fuscous at the metathorax and the median part of femora or with the greater part of metathorax, abdominal sternites and legs piceous. Pubescence light yellow, rather short, sub-erect and dense; punctation uniformly fine, close and impressed; the lateral margins of pronotum slightly rounded and the anterior angles acute and sub-rounded; prosternum a little longer than wide, slightly narrowing and raised anteriorly, sub-rounded at the apex; the sixth abdominal sternite slightly emarginate in female, spermatheca (fig. 17) expanded and rounded at the apical fourth, strongly bent in the middle and narrowed towards the base. Male not known.

Length 3·25 mm., width 3·0 mm.

Type in the British Museum; locality: South India, Munnar, feeding on *Icerya purchasi* Maskell, 1947 (Plant Protection Adviser, India).

Paratypes : One with the same data as the type in the Indian Museum (P. P. A.'s bequest), the other from the same locality and host, 22.i.1944 (*V. P. Rao*) in the author's collection.

Remarks. Close to *amabilis*, from which it may be distinguished by the presence of the black spots and by the structure of the genitalia.

Rodolia minima, sp. n. (figs. 8, 9, 19 and 20).

Body sub-hemispherical, rather obtusely rounded towards the apex; head and pronotum carmine-red, elytra for the most part like the pronotum but with the lateral margins and the apical third dark brown or piceous; underside usually carmine-red, metathorax sometimes dark brown; pubescence yellowish to light grey, short, dense and rather woolly in appearance; punctation fine and close, slightly less fine and rather more impressed on the elytra than on the head and pronotum; pronotum with the lateral margins slightly rounded and the anterior angles acute; prosternum nearly as wide as long, slightly narrow anteriorly and raised; sixth abdominal sternite emarginate in male and only weakly so in female; male genitalia as shown in figs. 8 and 9. The narrowed apex of penis curved downwards and the dentulation of sipho are characteristic; in female the ninth sternite narrowed towards the apex and rounded only on the external margin near the apex, spermatheca (figs. 19 and 20) broadly reniform and obovate in the lateral and front views respectively.

Length 2·35 mm., width 2·0 mm.

Type in the British Museum; locality: South India, Coty, feeding on *Icerya purchasi* Maskell, 5.v.1947 (Plant Protection Adviser, India).

Paratypes. Six with the same data as the type; of these, one is in the British Museum, three are in the Indian Museum (P. P. A.'s bequest), and two in the author's collection.

Remarks. On account of the different form of its sipho, this species does not appear to be closely related to any of the others dealt with here.

References.

Crotch, G. R. (1874). A revision of the Coleopterous family Coccinellidae. 311 pp.

Gorham, H. S. (1901). Stettin. ent. Ztg., **62**, p. 211.

Korschefsky, R. (1931). Coccinellidae I.—Coleopt. Cat., Berlin, **118**, 224 pp.

——. (1940). Ent. Blätt., **36**, p. 2.

Mulsant, E. (1850). Species des coléoptères trimères sécuripalpes.—1104 pp.

——. (1853). Opuscules entomologiques, **3**, pp. 205.

Sicard, A. (1909). Ann. Soc. ent. Fr., **78**, p. 117.

Weise, J. (1892). Ann. Soc. ent. Belg., **36**, pp. 16-30.

——. (1901). *Ibid.*, **45**, pp. 91–96.

——. (1912). Arch. Naturgesch., **78**, A12.

JASSID RESISTANCE AND HAIRINESS OF THE COTTON PLANT.

By F. R. Parnell, H. E. King and D. F. Ruston.
Empire Cotton Growing Corporation, Cotton Experiment Station, Barberton, South Africa.

E M V.

Contents.

	PAGE
Introduction	539
Local conditions and material	540
Determination of resistance	541
Determination of hairiness	543
Description of technique for recording hairs	546
Relationship between resistance and hairiness	547
Lower surface of leaf lamina	547
(*a*) Strains of *Gossypium hirsutum*	547
(*b*) Single plants of *G. hirsutum*	553
(*c*) Single plants of *G. barbadense*	554
(*d*) Single plants of hybrids	556
(*e*) Plants of different ages	559
Other parts of the plant	562
(*a*) Midrib	563
(*b*) Petiole and stem	566
General discussion and practical implications	567
Summary	570
Acknowledgements	571
References	572
Appendix.—Note on statistical treatment of data	572

Introduction.

The importance of Jassids (*Empoasca* spp.) as pests of cultivated cotton is widely recognised, particularly in those regions of Africa where cotton grows under rainfall, and in parts of India.

A description of the nature and extent of the damage caused to American Upland cotton, *Gossypium hirsutum*, in South Africa by *E. facialis*, Jac., has been given by Parnell (1927), who also presented conclusive evidence that the damage observed may, in fact, be attributed to this insect. In general the damage caused by this and other species elsewhere, on various types of cotton, would appear to be similar in nature.

The most satisfactory method of control of this pest on a large scale lies in the use of resistant varieties. The breeding of resistant types suited to South African conditions has been successfully carried out at Barberton (Parnell, 1935), and Jassid is no longer a pest of importance to the commercial crop. This breeding work was greatly facilitated by an observed relationship between hairiness of the plant and resistance to infestation—the hairier the plant the greater being its resistance. A guide such as this is of obvious value, since the plant breeder cannot always ensure the exposure of all his material to heavy and uniform Jassid infestation in order to judge resistance directly.

Successful breeding work can be carried out on *hirsutum* cotton, using general plant hairiness as a guide, with little knowledge of what constitutes the best type of hairiness, and of the exact relationship between hairiness and resistance. Such information is obviously, however, of considerable scientific value. Moreover, it becomes of practical importance in breeding work with cottons, such as certain species crosses, which show an extreme range in types of hairiness and in which the hairiness of stem, petiole and leaf are not closely related, as they are in *G. hirsutum*. If it can be shown that hairiness of a particular type on a particular part of the plant is most effective in conferring resistance, breeding for Jassid resistance may be modified to have as its immediate object the production of plants carrying this type of hairiness, with resulting economy of time and effort.

There have been reports from various quarters, particularly from India, which do not agree with experience at Barberton. Thus Husain and Lal (1940) stated that while nearly all resistant varieties of cotton are hairy, not all hairy varieties are resistant. Further, Lal (1941), referring to pubescence of leaves as a protective factor against Jassids, considered this factor to be of very doubtful utility. Again Afzal (1941) recorded that hairy plants could be highly susceptible, and sparsely hairy plants could show a marked degree of resistance. In an earlier review of the problem, Husain (1938) suggested that Jassid-resistant characters in the cotton plant are perhaps linked with hairiness and that hairiness itself may not be important. A more recent reference (Afzal & Manzoor Abbas, 1944) indicated that this view is still held, though Lal and Husain (1945) advocated a more critical approach to the use of hairiness as an indicator of Jassid resistance.

All the above references are concerned with *Empoasca devastans*, Dist., under Indian conditions and it does not follow that a relationship established for *E. facialis* at Barberton must apply. At the same time it is not safe to assume that the same relationship does not hold without detailed investigation, in which critical attention must be paid to the concept of " hairiness " and to the methods employed in assessing both hairiness and resistance. Hair length is clearly a most important factor which should not be neglected in any such investigation.

The purpose of this paper is to present the results of investigations carried out at Barberton, which establish beyond doubt that a connection does exist between hairiness and Jassid resistance. A method of analysing " hairiness " has been devised and information obtained on the type of hairiness that is most efficient in conferring resistance.

Local Conditions and Material.

The species of Jassid causing damage to cotton in South Africa is *E. facialis*, Jac. Under normal conditions at Barberton the nymphs do not appear in large numbers on any cotton until the crop is well beyond the seedling stage. The crop is planted on the spring rains, about November, after a cold and very dry winter period, during which most species of plants and insects remain dormant. By mid-season, however, the Jassid population on susceptible types normally becomes sufficiently heavy to result in serious damage, which increases as the season advances. The intensity of attack varies from season to season, but highly susceptible types are invariably heavily damaged and produce little or no crop : by the end of the season none but very highly resistant types remain free from symptoms of attack.

Under these conditions the use of a susceptible variety proved uneconomic and the local industry was threatened with extinction. The development of a resistant type to replace the original commercial varieties has completely changed the position. The present strain grown commercially, U.4/5143, does not go through the season entirely free from all damage to the leaf, but the crop is never affected to an appreciable extent.

Jassid is always present in the area and can be relied on to build up a heavy infestation on any susceptible type that may be planted. For this reason it has been possible to rely on natural infestation in the work described. In certain cases, steps have been taken to build up a high and uniform population by planting rows of a highly susceptible variety in alternation with rows of the experimental material.

A broad programme of introduction and hybridisation has enabled a very wide range of types to be studied under local conditions. The hybrid material from *G. hirsutum* x *G. barbadense* has been particularly useful in providing extreme types of hairiness not otherwise available. Brief descriptions of the types included in the various experiments are given later, but it should be noted that, in addition to specific experiments, all types grown at Barberton have been subjected to general observation from the points of view of resistance and of hairiness in order that no case of anomalous behaviour might escape investigation.

Determination of Resistance.

The problem of assessing resistance to Jassid is not merely one of drawing a distinction between " resistant " and " susceptible " strains. Over a range of types, the symptoms of Jassid attack vary widely in severity without any such clear cut division. Indeed with adequate material under observation, the range appears to be continuous between the two extremes of complete freedom from attack and virtual destruction of the plant.

It is necessary, therefore, to make use of some quantitative measure of relative resistance, and an arbitrary scale, based on visual assessment of degree of damage, has been in use in the breeding work from the earliest years. In this scale, as given on page 548, ten grades of damage are distinguished, ranging from " nil " to " very bad." The general healthy green appearance of the leaf of cotton at Barberton makes it easy to distinguish small differences in the symptoms, but the grades must be somewhat indefinite, varying with the observer and the material, and thus open to objection in a critical study.

An alternative is to use *infestation* as the measure of susceptibility and to base comparisons on counts of insects, instead of scores for damage. To do this narrows the sense in which the term susceptibility is used, and eliminates from consideration the potentially important elements of tolerance and power of recovery. Data are presented later which indicate that, within a body of material of the same general type, there is in fact a broad general agreement between scores for damage and counts of infestation. Apart from this, however, the investigations described here are mainly concerned with resistance to infestation, for which actual counts of insects are essential.

The nymphs are found almost exclusively on the lower surface of the lamina and, although they tend to seek cover when the leaf is turned over, they are not difficult to count accurately. In this they appear to differ from those of *E. devastans*, as reported by Indian workers (Verma & Afzal, 1940). Adults, on the other hand, almost invariably take wing when disturbed and are, in consequence, automatically excluded from the counts. Observations made by Parnell (1927) suggest strongly that nymphs, rather than adults, are the cause of most of the damage on cotton plants. Hence the omission of adults from the counts may be actually advantageous.

The workers in India (quoted above) appear to have made use of counts over the whole plant, recording up to 62 nymphs per plant at peak periods. Similar counts at Barberton have given figures up to 440 per medium sized plant of a highly susceptible strain of *G. hirsutum*. Counts on so large and indefinite an area of foliage are, however, not only quite unnecessary, but also unsound for accurate comparative purposes. Apart from large inherent strain differences in area of foliage, leaves of susceptible strains, exposed to Jassid for any length of time, become severely damaged. If not shed, such leaves soon cease to support nymphs. The " effective " area of two plants of the same size may, therefore, differ widely, that of the less susceptible being much the greater. With an incubation period of the order of eight days, nymphs are not to be found on very young leaves which have been exposed to oviposition for less than this period. For comparative figures it has been found most satisfactory to restrict counts to a chosen region of the foliage only. Main-stem leaves near the top of the plant are most convenient, omitting the topmost three as being too young. Any choice of comparable leaves, however, gives sound figures, if really old and senescent leaves are avoided. Counts at weekly, or half-weekly, intervals over a period of several weeks average out errors of counting and short term fluctuations.

The question arises as to whether the figures may not be made still more closely comparable by adjusting the actual counts, from a fixed number of leaves, for differences in leaf area. Such adjustments have been made but in general have little effect. Moreover, with very susceptible types, it appears that heavy infestation on a leaf before it is fully grown can result in premature cessation of expansion, and thus interfere with comparisons based on nymphs per unit area of foliage.

The period in the season during which counts are carried out is most important. Fig. 1 illustrates the general course of events at Barberton, in which infestation mounts to a peak as the season progresses, after which there is a marked falling off in the figures. This reduction in infestation, observed on selected leaves, normally takes place at a time when falling temperatures may be expected to reduce insect activity. The fall commences earlier, however, on types which have suffered severe damage and may be attributed in part to changes in the condition of the foliage, the effects of which extend to any leaves which may be chosen for counting. With all types, a point is eventually reached when vegetative growth virtually ceases and there is no longer any attractive fresh foliage. This cessation of growth is partly dependent on the date of sowing but it is also influenced by falling temperatures, and the setting of fruit. The figures illustrated are from a replicated experiment in season 1945/46. The build-up was steady and rapid as the plants were late sown and adjacent to earlier sown cotton on which a population was already established. Roughly similar figures have been obtained, however, in other experiments and other seasons (see Parnell, 1928, pp. 57, 60 and MacDonald and others, 1944, p. 28). It is clear that the comparative infestation on different types is roughly the same for increasing degrees of general infestation until the more susceptible types reach a peak. Beyond this point differential " leaf damage " effects appear and further counts are liable to give anomalous results.

Distribution of infestation in a large area of cotton is not entirely uniform. On occasion, a clear " drift " may be detected, sometimes traceable to a definite " pool " of insects on some earlier sown, or particularly susceptible, type. Even quite resistant types may show appreciable infestation when grown adjacent to very heavily infested areas, while remaining almost free under more normal conditions. This " bombardment effect " is illustrated by data given in the 1942–43 Annual Report of the Station, p. 29 (MacDonald & others, 1944). With precautions, however, such irregularities may be rendered relatively unimportant, particularly when replicated experiments are possible. With equal exposure to infestation, fluctuations in counts from plot to plot are roughly proportional to the general level of the population and a logarithmic transformation of the crude figures has been found appropriate in the analysis of the data (see Appendix).

Determination of Hairiness.

Hairiness of the cotton plant is a complex of a number of factors, the most obvious being the density, length and distribution of the hairs on the plant. In material studied at Barberton the number of hairs per square centimetre has been found to range from none to over thirty thousand and the mean length from a quarter of a millimetre to nearly two millimetres. Hairs may occur in abundance on stem, petiole and both surfaces of the leaf (including midrib), or may be almost entirely confined to the lower surface of the leaf lamina.

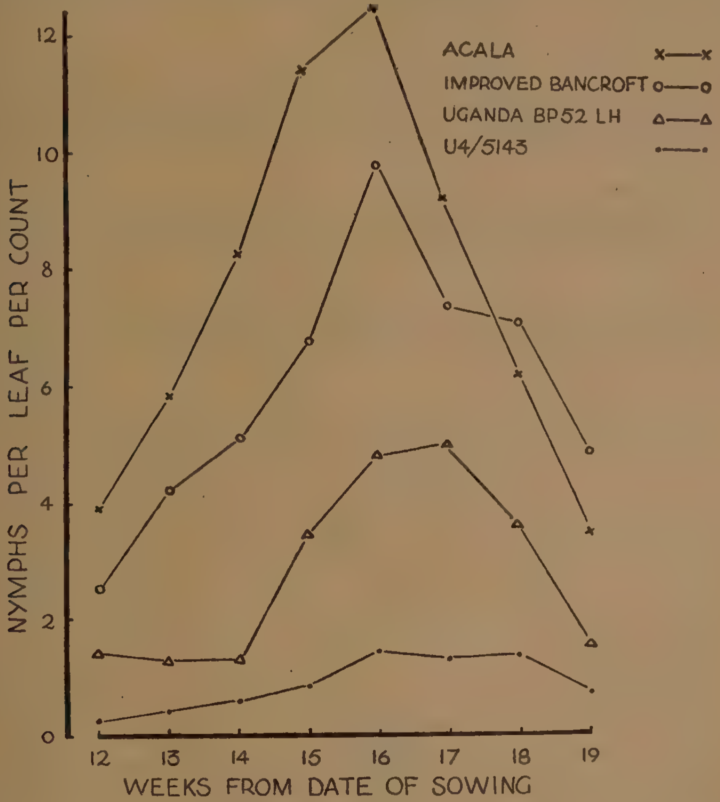


Fig. 1.—Jassid infestation week by week through the season on four strains of *G. hirsutum*.

Furthermore, both density and mean length of hairs, on any one part of the plant, are themselves complex in nature. A particular density may be made up of uniformly scattered hairs, or of groups of hairs with bare areas between. The length of individual hairs may be comparatively uniform, or may vary very widely from hair to hair. In addition, variation occurs in the straightness and erectness of hairs, and most probably also in stiffness or flexibility. Some of the characters are to a considerable extent interconnected. Thus, when a number of hairs spring from a common origin, forming a " bunch," they tend to be much shorter, and less erect, than single hairs standing alone. Some illustrations of the wide variation in nature as well as extent of hairiness will be found in figs. 2, 7, 8 and 9.

It is evident that a single precise and comprehensive definition of "degree of hairiness" cannot easily be formulated. An assessment taking into account every observable variation in nature and distribution of hairs is clearly not practicable, and some choice, amongst the many measurable characteristics, is inevitable. Having regard to the purpose of the investigations, the obvious criterion in making a choice is the degree of association shown with resistance. It does not, of course, follow that the same measure, or combination of measures, will give the closest association in every case. Many alternatives must be tried over a wide range of material before a decision is reached.

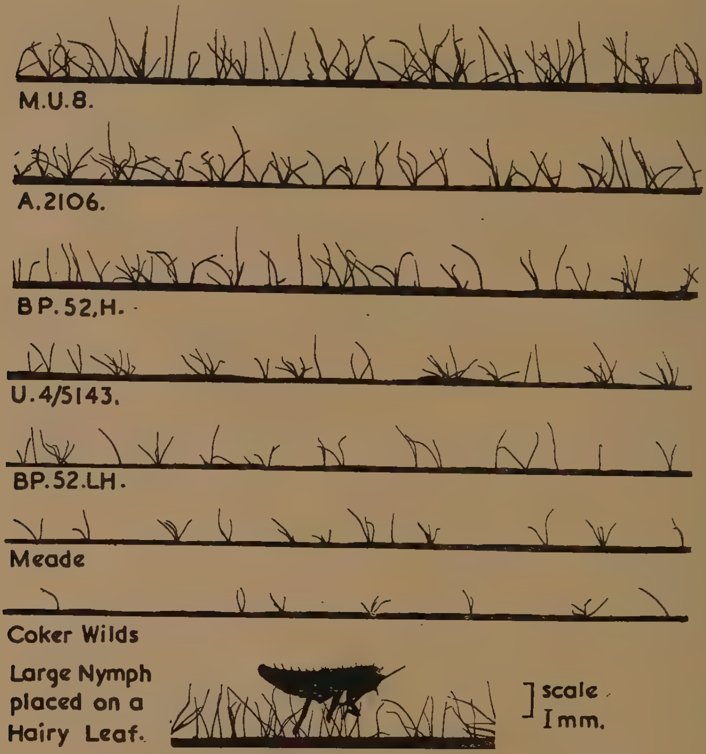


Fig. 2.—Range of lamina hairiness on varieties of *G. hirsutum.*

Silhouettes (×8), of sections 1·4 mm. wide; each strip portrays the hairs carried on approx. 18 sq. mm. of lower surface of lamina. The sections illustrate the data given in Table I for season 1943–44. M.U.8 is almost immune and Coker Wilds highly susceptible to Jassid.

The silhouette of the nymph indicates the size of the insect relative to the length of the leaf hairs on M.U.8.

If attention is confined to varieties of *G. hirsutum,* degrees of hairiness, clearly related to resistance, are unmistakably recognisable in the field without recourse to any counts or measurement of hairs. The classification of strains and single plants by eye has, at Barberton, given most consistent results and was indeed the method successfully employed in the production of Jassid-resistant strains.

In a large number of *hirsutum* types examined the general " style " of hairiness is consistent (fig. 2), and variations in density and length of hairs are positively correlated. In general a high degree of hairiness, as judged by eye, is in fact a combination of good density and good length, while poor hairiness commonly involves a lack of both density and length. Moreover, degrees of hairiness of stem, petiole and leaf, although not directly comparable, are in general related and the hairiness of the plant can, therefore, be judged as a whole.

With other types, however, and particularly in material obtained by hybridisation between *G. hirsutum* and *G. barbadense,* eye-judgment of degree of hairiness is far more difficult. Very much higher densities are met with and a marked negative correlation between density and length of hairs may occur. Furthermore, hairiness of leaf lamina is not necessarily associated with hairiness of other parts of the plant. To cover such cases, and to provide quantitative data for critical examination, hairiness of separate parts of the plant must be studied individually and more exact methods of assessing hairiness must be developed. Such studies have been made in great detail on a number of distinct sets of material from three seasons' experiments, but the bulk of the work has been concentrated on hairs on the lower surface of the lamina.

No detailed study has been made of the effect of hairs on the upper surface of the lamina.* Since it quite frequently happens that this part is almost completely glabrous, even in highly resistant plants, it may be assumed that hairiness here is of little or no direct importance.

Details of the results are given later, but briefly it has been found that counts of total density, taken alone, are only effective in material showing a strong positive correlation between density and length. Almost any measure which takes length of hairs into account shows a significant correlation with infestation. Mean length alone is quite useful with certain material, but is not satisfactory when the range in density and " style " of hairiness is wide. Density and mean length taken together (in a multiple regression equation) are effective, but have not proved the most satisfactory of all measures tried. When very short hairs are unduly numerous and occur as an undergrowth to the longer hairs, they inflate the figure for density and bring down the mean length. Higher correlations with infestation are obtained when such short hairs are ignored.

The vertical " height " of the hairs, measured at right angles to the lamina surface, is much more easily and rapidly obtained than the true length hair by hair, and has been found at least as useful. A measure which combines features of both height and density in a single figure has obvious advantages. Such a figure is given by *counts of hairs per unit area, exceeding a specified height.*

As a result of trials covering more than a score of different measures and combinations of measures, counts of hairs exceeding heights of ·3 mm. to ·5 mm. have been found most universally satisfactory for reducing all styles and degrees of *lamina* hairiness to a simple numerical value, giving high correlations with infestation.

It is not to be assumed, however, that other features of hairiness are without influence on resistance, nor that this particular range will cover the best height for every individual case. A study of hairy types under more severe conditions of infestation than are experienced at Barberton might well show that hairs at much greater heights give added resistance. It seems clear, however, that a certain minimum value for hairs exceeding a height of ·5 mm. confers almost complete immunity under quite stringent conditions, virtually regardless of all other features.

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*Records of hairs on the upper surface of the lamina are provided automatically when lamina strips are photographed. In the figures, however, only the lower surface has been reproduced, the upper surface being screened off.

Counts at lower heights are necessary to distinguish between plants carrying no hairs at a height of ·5 mm., and yet differing in degree of susceptibility.

Description of Technique for Recording Hairs.

The following notes give briefly the main points of the technique used in obtaining records of hairiness. In view of the extreme variability found in all material from cotton plants, the aim throughout the work has been to devise methods by which large numbers of samples could be handled, rather than to strive for extreme accuracy. Using the methods described, determinations of hairiness have been made on some 20,000 specimens in the course of four seasons.

Leaf Lamina.

The sample used is a narrow strip cut from the lamina. Widths of 0·5 to 2·3 mm. have been used with 1·4 mm. as the final standard for most of the work. The strips are cut with a pair of safety razor blades separated by a metal plate of the required thickness and firmly clamped between two supporting strips of stiff metal. The upper surface of the leaf is laid against a smooth wooden block and a drawing cut made with the cutter pressed against the lower surface. This may be done in the field without removing the leaf from the plant.

A standard portion of the leaf is sampled, the cut being made between, and parallel to, the secondary veins of the central lobe running out at an angle from the midrib to the lateral edge of the lobe. On all but exceptionally small leaves this gives a strip exceeding 25 mm. in length.

The cut strips may be examined at once or may be preserved in 50 per cent. alcohol until required.

For examination, or photographing, the strip is mounted on a small wooden holder which enables the hairs to be viewed in profile. The strip is drawn taut and held in position by two small metal clips spaced 20 to 25 mm. apart. Once mounted it is dealt with at once before drying and shrinking.

A permanent record of all the features visible in profile is obtained by inserting the holder in a photographic enlarger in a dark room, and projecting a silhouette at a magnification of x10 on to bromide printing paper. A small aperture (f.32) is used to give depth of focus, with a light source sufficiently intense to keep the exposure time short (6 to 8 seconds).

On development the paper provides a " negative " (white hairs on a black background) which may be examined direct, or used to print positives if copies are required. The hairs portrayed in this way are easily studied with the naked eye or with the help of a reading glass, if dense and short. With a lamina strip width of 1·4 mm. a length of 178 mm. on the print is taken to represent 25 sq. mm. of lamina surface.

To count the number of hairs exceeding a specified vertical height, the paper is held beneath a sheet of glass carrying a fine horizontal line at the required distance (x 10) from, and parallel to, the surface of the lamina. Hairs not crossing the line are then ignored, whether erect or spreading. An opaque band on the glass may be used to obscure the unwanted hairs.

Midrib.

Reference will be made (p. 562) to two alternative methods of observing midrib hairs.

Longitudinal silhouettes are quite simply obtained by mounting a length of midrib in the same manner as a strip of lamina. Records made in this way give figures for the number of hairs projecting a specified distance beyond the extreme outline of the midrib, but cannot yield counts of total density over the curved surface.

Cross sections are mounted and photographed by the same method, being cut with two " tags " of lamina attached to provide means of clipping into the holders.

Relationship between Resistance and Hairiness.

LOWER SURFACE OF LEAF LAMINA.

(*a*) Strains of *Gossypium hirsutum.*

Experiments with strains of *G. hirsutum* (American Upland Cotton) were carried out in two successive seasons. Thirteen strains were included in one experiment in 1943-44, and nine of these, together with one additional strain, appeared in a second experiment in season 1944-45. The choice of strains was made from material available at Barberton, to include representatives of various degrees of resistance to Jassid. The majority were importations to the Station and not the result of local breeding work. The strain P.43 F was included expressly on account of reports that its behaviour did not support a belief that hairiness implied resistance.

Brief details of the individual strains are as follows :—

Importations from America.

Acala. A strain of long standing in the U.S.A. The least hairy and most susceptible Upland type grown at Barberton.

Tidewater and Coker Wilds, No. 7. Long-stable, high quality types, also very susceptible.

Meade. A high quality type, rather less susceptible.

Improved Bancroft. An old variety, at one time extensively grown in South Africa, but discarded as too susceptible to Jassid.

Importations from India.

M.U.8. A malwa Upland type from Indore, noted for a high degree of hairiness.

P.43F. A Punjab strain, reported to be very hairy but not always resistant to Jassid.

C.9363. A Barberton selection from " Cambodia ", less hairy than the original importation from South India.

Importations from Uganda.

BP.50/3. A high quality strain giving good resistance at Barberton.

BP.52 H and BP.52 LH. Approximately true-breeding sub-strains, with greater and less hairiness respectively, separated at Barberton from the high quality Uganda strain, BP. 52.

Locally-bred Strains.

5143. A sub-strain of the Jassid-resistant type U.4, bred at Barberton and now in general cultivation in the area.

8161. Another U.4 strain, slightly earlier and very slightly less resistant than the above.

A.2106. A hybrid strain from U.4/5143 x Cambodia but of better hairiness than either parent.

The 1943-44 experiment included all the above strains except Improved Bancroft. Four replications were arranged in randomised blocks, the individual plots consisting of two rows, each of 25 plants. Each plot was separated from its neighbours by a row of fully susceptible (Acala type) cotton, with the object of exposing all strains to a high and uniformly distributed population of Jassid.

Counts of nymphs were made at weekly intervals on five plants in each plot, the count being confined at first to four and later to five full-grown main-stem leaves at fixed positions relative to the topmost node on the plant. Through the season, therefore, the zone in which the counts were made moved up the plant with the growth of the main stem. The choice of plants was confined to reasonably well-grown specimens, avoiding obvious off-types and edge plants, but was in other respects " random ". Changes were made from time to time to avoid excessive handling effects and to maintain the full number of plants.

At approximately 16 weeks from date of planting, samples of the lamina of one main-stem leaf per plant, from four plants in each plot, were taken for determination of hairiness. These plants were chosen from those on which Jassids were then being counted, and the leaf sampled, the eighth from the top, was from the part of the plant on which counts were being carried out at that time.

The counts of nymphs showed considerable fluctuations through the season, with the relative infestation of the different strains changing to some extent. In particular, the figures for those strains carrying the highest early infestation fell towards the end of the season, while counts on resistant strains were still rising. Strain comparisons are, therefore, dependent to some extent on the period when counting takes place. The most useful period for a general comparison of strains, and for correlation with hair data, was considered to be the four weeks immediately prior to the sampling for hairs and the four following.

Over this period of eight weeks the total number of leaves on which nymph-counts were taken was 720 per strain, but the values given in Table I were obtained from the *geometric* means of the four plot-totals of 180 leaves each. These have been converted to nymphs per 1,000 leaves to facilitate comparison with other experiments.

The hair figures were obtained from the examination of 180 sq. mm. of lamina surface per strain, by use of the photographic technique described above, except for the strains Tidewater and Acala. For these, the very few hairs present on the leaf strips were counted but not photographed, as it was obvious that extremely few would have appeared in a count at a height of ·3 mm.

Twenty-one weeks from the date of planting, viz., shortly after the end of the period referred to above, a visual assessment was made of the symptoms of Jassid attack on the foliage. Each plot was judged as a whole and given a score indicating relative severity of attack, as follows :—

Nil—No visible symptoms ... ... — 0
Very slight effect—Slight yellowing on the edges of occasional leaves ... ... — 1
Slight effect—Yellowing more pronounced ... ... — 2
Obvious effect—Yellowing of leaf edges general on plant; no real damage ... ... — 3
Very slight damage—Slight reddening, with first sign of curling on occasional leaves ... ... — 4
Slight damage—More pronounced reddening and curling on plant generally ... ... — 5
Moderate damage—Very definite reddening and crumpling, but plant mainly green and leaf functioning ... ... — 6
Rather bad damage—Leaf mainly red and crumpled; beginning to dry up ... ... — 7
Bad damage—Leaf mainly dried up ... ... — 8
Very bad damage—Plant leafless ... ... — 9

The average of the scores for the four plots of each strain is given in Table I.

TABLE I.

Lamina hairs and Jassid infestation on strains of *G. hirsutum* in two seasons.

Strain	Key No.	Season 1943–44					Season 1944–45		
		Lamina Hairs per sq.cm.			Nymphs per 1,000 Leaves	Score for Effect	Hairs per sq. cm. exceeding heights of		Nymphs per 1,000 Leaves
		Total (all heights)	Exceeding heights of ·3 mm.	·5 mm.			·3 mm.	·5 mm.	
M.U.8 ...	1	575	465	323	3	0	415	276	0
A.2106 ...	2	508	346	163	40	0·25	—	—	—
BP.50/3 ...	3	574	343	137	78	2·25	—	—	—
P.43 F ...	4	403	266	157	109	1·25	234	117	65
BP.52 H ...	5	374	252	125	106	3·13	214	115	90
C.9363	6	416	319	152	209	4·25	229	72	132
U.4/5143 ...	7	367	215	79	105	3·38	169	56	264
U.4/8161 ...	8	436	252	88	230	4·33	157	49	227
BP.52 LH ...	9	246	149	72	525	5·38	104	33	1,463
Meade	10	210	120	40	1,498	6·75	97	22	2,460
Improved Bancroft ...	11	—	—	—	—	—	57	4	3,517
Coker Wilds ...	12	92	47	8	3,065	7·25	—	—	—
Tidewater ...	13	45	(0)	(0)	3,137	7·50	—	—	—
Acala	14	9	(0)	(0)	3,838	7·38	0	0	6,942

The 1944–45 experiment was similar in design but with ten strains and two replications only. Counts on five consecutive main-stem leaves were made on nine plants per plot twice a week, with the same leaves and plants retained over the period of seven counts (22 days). At the end of this period these same leaves were sampled for hairs, two of the recorded leaves being taken from each counted plant of the majority* of the strains. The samples, therefore, represented a considerable proportion of the actual leaves on which the nymphs were counted and the infestation records refer only to a small chosen zone of the plant and to a short period in the season.

The data from this experiment are given alongside those of the 1943–44 experiment in Table I. The infestation figures were obtained from 630 leaf counts per strain, the geometric means of plot totals (315 leaf counts) again being expressed as nymphs per 1,000 leaves. The hair figures for this second experiment were obtained from the examination of 900 sq. mm. of lamina per strain, except for the four types mentioned in the footnote where a 300 sq. mm. sample was used. Wider leaf strips were used, as the number of hairs present at heights of less than ·3 mm. was not required. No record of "Jassid effect" was made in this experiment.

It is at once evident from the figures of Table I that a most striking degree of association exists between "infestation" and "lamina hairs" when length of hairs is taken into account. The consistency with which the count of infestation corresponds with the figure for hairs is brought out in fig. 3, in which infestation is plotted on a logarithmic scale against number of hairs per sq. cm. exceeding a height of ·3 mm.

It will be seen that, in each season, all the strains included fall very well into line, and the existence of a very strong negative correlation is quite unmistakable. Using a logarithmic transformation for the infestation data, the coefficients of correlation, with hairs exceeding ·3 mm. in height, reach the extremely high and significant values of –·962 in the 1943-44 experiment and –·987 in 1944-45.*

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* For the more hairy types (Nos. 1, 4, 5 and 6) only six plants were sampled, three per plot, to reduce the labour of counting hairs.

In both seasons the count of hairs at a height of ·3 mm. proved the most satisfactory single figure for distinguishing degrees of resistance over this range of varieties. It is clear, however, from a study of the other figures in Table I that this choice is a compromise, and that for certain comparisons it is not the most appropriate. Thus, a clear difference in resistance is shown by the strains A.2106 and BP. 50/3 which gave almost identical hair counts at this height. The greater resistance of A.2106 is to be attributed to the added number of hairs at a height of ·5 mm. At the other end of the scale, Tidewater shows some reduction in infestation compared with Acala. Counts of total hairs reveal some difference in hairiness, but neither strain has sufficient long hairs to give appreciable densities at a height of ·3 mm.

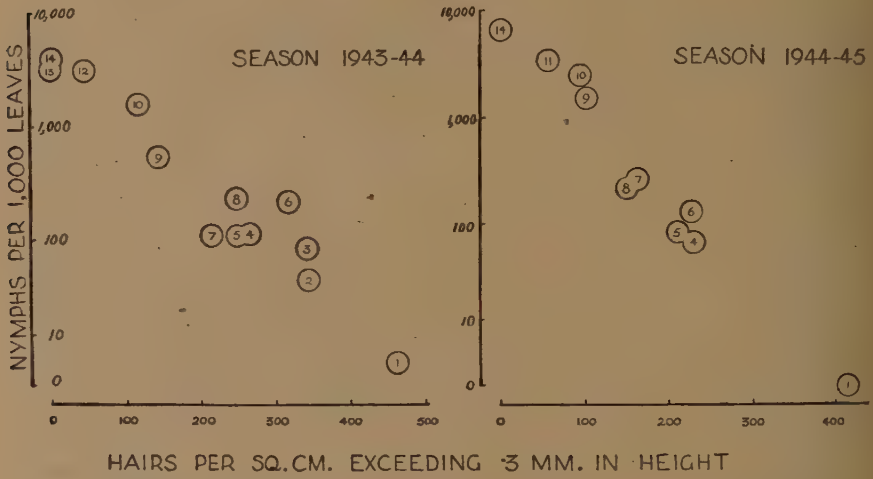

Fig. 3.—Jassid infestation and Lamina hairiness of strains of *G. hirsutum* in two seasons. (For key to numbers representing strains see Table I.)

In general, the figures show clearly that counts of " total density " with " length " disregarded, do little more than distinguish between very large differences in hairiness and would be a misleading guide to " resistance " when comparing heavy strains among themselves. Thus, strain 8 (U.4/8161) has a higher *total* density than strains 4, 5, 6 and 7, while strain 3 gave the same figure as strain 1 but is very definitely less resistant.

The desirability of choosing a height appropriate to the material has already been mentioned. Where the range is very wide, some advantage is gained by combining counts at two heights in a multiple repression equation, but with the data in Table I the gain is small, and the simple relationship of infestation with a count at a single height is more than sufficiently convincing.

The general agreement between the two seasons' results is highly satisfactory but a number of differences occur and are worthy of comment. The changes in experimental technique, in the second season, were intended to give a more accurate comparison of infestation and hairiness on chosen foliage of particular plants. The result has been an even closer relationship than in the earlier experiment. In particular it may be noted that strain 6 (C.9363), which showed a wide plant-to-plant range in hairiness, falls better into line in the second season. It is probable that in 1943–44 the figures for hairs were not properly representative of the hairiness of the plants yielding the infestation data.

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*At the most stringent odds of P=·001 the magnitude required for significance is ·801 in 1943–44 and ·872 in 1944–45.

It will also be seen that the general level of hairiness (as indicated by counts at heights of both ·3 and ·5 mm.) is very definitely lower in the second season for all strains, except Acala, common to the two experiments. Growth conditions were much less satisfactory in 1944-45, and the foliage sampled was developed during a drought. Extensive observations have since shown that growth conditions very markedly influence hair development, and that differences of this magnitude from season to season and from crop to crop are common.*

Actual figures for infestation in 1944–45 cannot be compared directly with those for the previous season, since the method and period of recording were different. The two seasons' figures serve to illustrate the variability to be expected in work of this nature but, at the same time, are mutually supporting as regards the general relationship between hairs and infestation. The drop in hairiness shows some variation from strain to strain but in general the relative hairiness and the relative infestation are substantially unchanged.

A further point of interest in Table I is the degree of correspondence, in the 1943–44 experiment, between infestation and " score for effect ". It is clear that, in general, the severity of symptoms of Jassid damage follows closely the relative infestation figures obtained by counting nymphs. The symptoms varied from " nil " on M.U.8 to " rather bad to bad " damage on Tidewater.

It will be noted that the infestation recorded on U.4/8161 was, in both seasons, less than one nymph per four leaves. This very light infestation tallies with the score for Jassid effect in 1943–44—equivalent to little more than " very slight damage ". These results were obtained from plants exposed to a large population of Jassid, bred up on adjacent susceptible material. Strains with greater hairiness than U.4 gave even lower figures and, in consequence, the differences between them are small and, superficially, may appear of very little practical importance and of low accuracy.

It is of value, therefore, to observe the reaction of the more resistant types later in the season. Further counts were made on the 1944–45 plots some weeks subsequent to the period covered by the data already presented. At this later period Jassids were extremely abundant, but the more susceptible types, already suffering severe damage, were rapidly losing their attractiveness. The data given below for the four most resistant strains provide a striking comparison with the earlier counts. The figures are given as nymphs per 1,000 leaves and (in brackets) as infestation relative to that on C.9363.

Strain	Earlier Period	Later Period
C.9363	132 (1·00)	2,398 (1·00)
BP.52 H	90 (0·68)	1,480 (0·62)
P.43 F	65 (0·49)	997 (0·42)
M.U.8	0 (0·00)	15 (0·01)

It is clear that the earlier counts did, in fact, bring out the *relative* differences in resistance with considerable accuracy in spite of the extremely low level of infestation. The general rise in counts on these strains was very marked indeed (approx. 1 : 17) but each showed a roughly proportional increase.

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*An example of the influence of conditions on hairiness will be found in the annual report of the station for season 1944/45 (MacDonald and others, 1946). Further data are being prepared for publication.

Proportionate changes are in accord with general experience in studies of insect populations (Williams, 1937) but for these to occur it is necessary that the foliage should remain in a comparable condition from strain to strain. It has already been pointed out that discrepancies appear in varietal comparisons when damage intervenes.

Over the same interval the infestation on U.4/8161 rose in the ratio of 1 : 13, giving almost 3 nymphs per leaf, a figure associated with considerable damage (*cf*. Coker Wilds in Table I). It must be emphasised that this figure, late in the season with very heavy Jassid attack deliberately fostered, is quite unrepresentative of U.4 cotton at Barberton. It provides however, an example of the need, under extreme conditions, for a higher standard of hairiness. M.U.8 with equal exposure to infestation remained virtually free.

An interesting change in relative resistance is revealed when infestation is recorded on individual leaves, commencing before they attain full size. Comparisons between strains may then be made using figures from part-grown and full-grown leaves separately. The data given below (taken from the 1944-45 experiment) illustrate results obtained in comparing the virtually hairless Acala strain with others carrying some lamina hairs. The figures given are for nymphs per 1,000 leaves, on two occasions, when the leaves were approximately " half-grown " and " fully-grown " respectively.

	Days from first start of unfolding	
	14 (half-grown)	28 (fully-grown).
Acala	4,889 (1·00)	13,711 (1·00)
Bancroft	1,437 (0·29)	8,229 (0·60)
Meade	804 (0·16)	5,961 (0·44)
BP.52 LH	353 (0·07)	3,334 (0·24)

The figures in brackets give the relative infestation on the basis Acala—1 on each occasion.

With a marked rise in Jassid population, the counts on the four strains are related to one another in a different proportion from that previously exhibited, and it is evident that their relative suspectibilities have changed appreciably.

The examination of leaves at different stages of expansion has shown that the full complement of hairs, of full length, is present at a very early stage, and that subsequent expansion results in a *reduction in density* proportional to the increase in leaf area (King & Ruston, 1945). Thus, a hairy leaf should decrease in resistance as it expands : on the other hand, Acala, which carries no hairs, should not show the same decrease. The figures given are in agreement with expectation. Though they do not indicate directly that susceptibility of Acala has remained unchanged, they show clearly that the resistance of the hairy strains, in comparison with Acala, has fallen.

It may be noted that the figures in brackets for the second count approximate fairly closely to the *square roots* of corresponding figures for the first count, a result which suggests that the resistance of each of the three hairy strains, in comparison with Acala, has been approximately *halved** between the two counts. It thus appears that the susceptibility of Acala has remained unchanged while

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*It has already been shown that a linear relationship exists between hairiness and the *logarithm* of infestation figures.

the resistance of the hairy strains has been halved, due to the leaf doubling in size and thereby halving the hair density.

In a strain showing a very low degree of hairiness on the mature leaf, the young expanding leaf may be hairy enough to delay the onset of infestation and hence to postpone the infliction of damage. It has, in fact, been generally observed that fully susceptible and moderately resistant strains do show a difference in rapidity of damage on the young leaves.

(*b*) Single Plants of *G. hirsutum*.

If the relationship between lamina hairiness and Jassid infestation, displayed when *strains* are compared, is a direct one of cause and effect, it should appear also when single plants within a strain are compared, provided the plant-to-plant range in hairiness is sufficiently large.

The majority of the strains included in the 1944–45 experiment (described in the previous section) did show a wide range in hairiness. Quite marked plant-to-plant variations in infestation also occurred, although, in the case of the more hairy strains, differences were not conspicuous until late in the season. M.U.8 and Acala proved exceptions, the former being regularly too resistant, the latter too susceptible, to show appreciable differences in infestation.

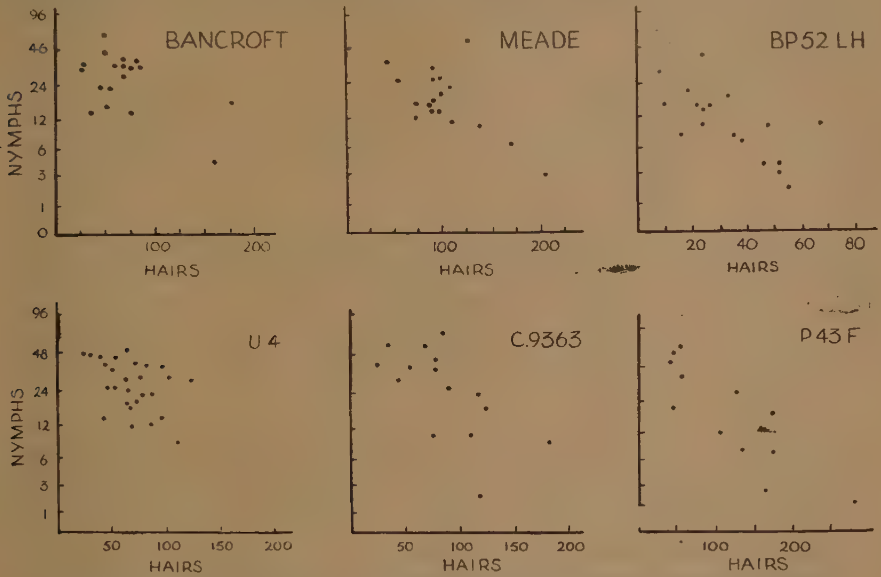

Fig. 4.—Jassid infestation and lamina hairiness of single plants within six separate strains of *G. hirsutum*. Counts of nymphs expressed as per 10 leaves throughout. Hairs per sq. cm. at a height of ·3 mm. for Bancroft and Meade, and at a height of ·5 mm. for the remaining four strains.

Data for each of the remaining eight strains were analysed separately, plant by plant, although in some cases the plants available were reduced, by casualties, to an inadequate number. It should be noted also that, in contrast with results based on means from a replicated experiment, the single-plant analyses are subject to enhanced errors from two sources. The sampling error of the figures for hairiness becomes of greater consequence, and the likelihood that all the plants were not equally exposed to infestation introduces a potentially serious disturbance, at least when comparatively small differences in susceptibility are under investigation.

In spite of all sources of error, however, every one of the analyses yielded a negative coefficient for the correlation between hairs and log. infestation. In all but three cases the significance exceeded P = ·05, the non-significant cases being BP.52 H, for which data from only ten plants were available, and the two U.4 strains, in which the range in hairiness was comparatively small. Even within U.4, however, the relationship became clearly significant when the data for 5143 and 8161 were combined in a single analysis with increased degrees of freedom.

The results are illustrated graphically in fig. 4. It should be noted that the actual levels of infestation are not comparable over the whole set. For the less hairy strains the counts were confined to the earlier part of the season, before the plants were seriously damaged by Jassid, while for the more hairy types the figures were obtained from later counts. It will be seen that, although the points are somewhat widely scattered, the same general relationship appears in each case.

It is of particular interest to note that the relationship holds within the strain P.43 F. from India. It is clear that a proportion of the plants within this strain are not fully resistant when exposed to heavy infestation, but this plant-to-plant variation is explicable by reference to the very considerable range in degree of hairiness of the lamina.

(c) Single Plants of *G. barbadense.*

Although Jassid-resistance in strains of *G. hirsutum* has been the main interest at Barberton, it was considered important to determine whether a similar relationship between hairs and resistance existed in the species *G. barbadense,* some commercial varieties of which, e.g., " Sea Island " and " Egyptian " cottons are susceptible, while at least one, " Tanguis ", is resistant.

The style of hairiness is notably different from that of typical *G. hirsutum.* Single long erect hairs are rare and bunches of many hairs (sometimes up to 12 or more) spreading from a common base, make up the bulk of the cover. Although more spreading and tangled, and appearing shorter, the actual lengths are of the same order as in *G. hirsutum.* The total density varies very widely, but may be so high that much narrower leaf sections are necessary for its determination. Counts of hairs reaching heights of ·3 mm. and upwards, however, were obtained by the same technique as that employed for *G. hirsutum* varieties.

Varieties of *G. barbadense* cotton are not successful at Barberton. Their long growing period and late cropping are in sharp contrast with the earliness of the *hirsutum* types, which do well in the area. " Sea Island " cotton and some strains of " Egyptian " (imported from the Sudan) give good vegetative growth, but fruiting is delayed until Jassid damage becomes severe, resulting in extremely low yield. They are also subject to heavy damage by " blackarm " (*Xanthomonas malvacearum*). " Tanguis ", on the other hand, after a very short period of good early growth, ceases to grow altogether, although not attacked by Jassid.

These factors make direct comparison with *G. hirsutum* for Jassid-resistance unsatisfactory, since the growth and condition of the plant appear to have some effect on attractiveness to Jassid. Moreover, as already mentioned, the development of hairs is influenced by conditions affecting the growth of the plant, and, apart from local climatic effects, the period in the season at which full hairiness is attained differs very markedly between the two species.

For these reasons, specific experiments have been limited to a study of single plants of a few types giving fair vegetative growth. Although the extremely hairy Tanguis variety had to be excluded, a wide range of hairiness (including some plants approaching Tanguis in degree of lamina hairiness) was available amongst selections made from Sudan strains of Egyptian cotton.

The results of an experiment with 23 plants are illustrated in fig. 5. The counts of nymphs and of lamina hairs were obtained in a manner similar to that for other experiments already described, and the infestation is again plotted against hair counts at heights of ·3 mm. It will be seen that the infestation, which shows a wide plant-to-plant range, may be predicted from figures for lamina hairs with much the same accuracy as in the case of *G. hirsutum* single plants.

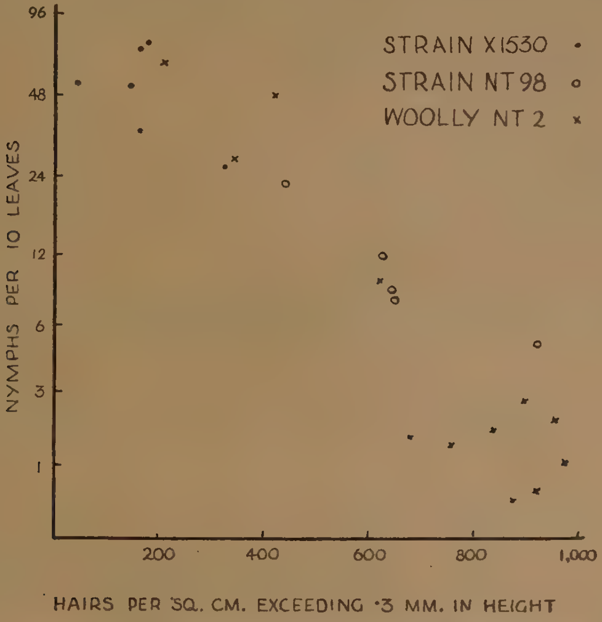

Fig. 5.—Jassid infestation and lamina hairiness of single plants from three strains of *G. barbadense*.

A "varietal" comparison can be made between the two groups of plants at the opposite extremes of the range in hairiness. This is given in the figures below, which also includes, for a rough comparison, mean figures for a group of U.4/5143 (*hirsutum*) plants, grown alongside and recorded over the same period.

Group of plants	Lamina hairs per sq. cm. at heights of			Nymphs per 1,000 leaves
	0·3 mm.	0·5 mm.	0·7 mm.	
Eight plants of "Woolly NT 2"	865	283	49	135
Eleven plants of U.4/5143 ...	174	43	9	1,358
Six plants of X 1530	167	33	2	4,926

The counts were made late in the season, when the general level of infestation to which the plants were exposed was very high, as indicated by the count given by U.4/5143. The low hair figures for this strain are comparable with those in Table I for the same season (1944-45), when growth conditions were unfavourable.

It will be seen that the figures form a series in which the *hirsutum* strain, U.4/5143, falls approximately into place between the two groups of *barbadense* plants. The very marked resistance of " Woolly NT 2 " is strikingly shown, but, in comparison with usual figures for *hirsutum* strains, an even higher degree of resistance might have been expected in view of its high hair counts at heights of ·3 and ·5 mm. A partial explanation might lie in the small number of hairs reaching greater heights than ·5 mm. In comparison with the figure of 49 per sq. cm. at a height of ·7 mm. given above, the figure for M.U.8 would be in the neighbourhood of 140. A further reason for the lack of full immunity is discussed in a later section dealing with hairiness of midrib.

(*d*) Single Plants of Hybrids.

When progenies from crosses between varieties of the two species *G. hirsutum* and *G. barbadense* segregate, an extremely wide range of types of lamina hairiness is obtained. Plants occur in which both density and length greatly exceed that of the more hairy (*G. hirsutum*) parent, together with others almost completely devoid of hairs. Almost every conceivable combination of length and density may be found, in conjunction with a wide range in " style " of hairiness (degree of bunchiness and erectness, etc.).

There is, however, considerable correlation between the different characteristics, with the result that some combinations are abundant and others rare. In particular, plants carrying only a poor density of short hairs are very numerous *, while, amongst the more hairy plants, very high densities combined with low mean

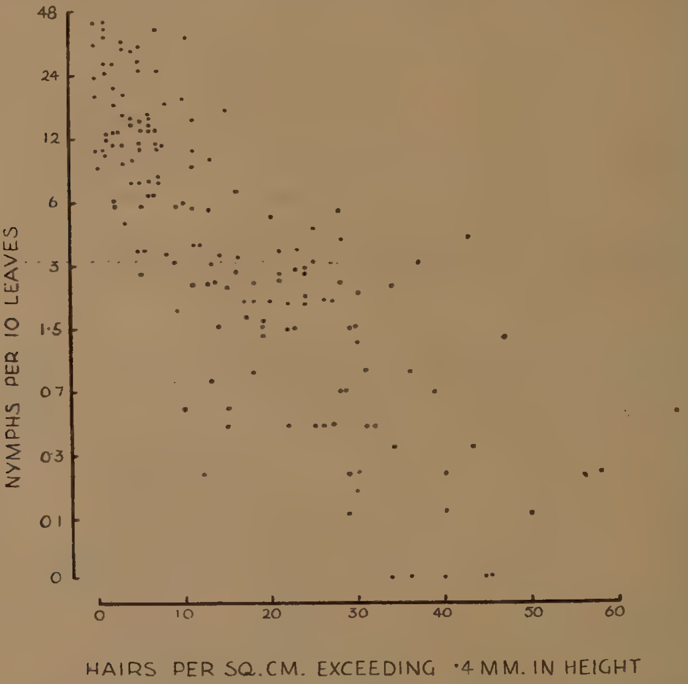

Fig. 6.—Jassid infestation and lamina hairiness on 155 hybrid single plants.

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*Over two thirds of the plants in an F_2 of U.4 × Sea Island carry such a poor cover that they are severely damaged by Jassid quite early in the season at Barberton.

lengths tend to predominate. For this reason, studies in Jassid resistance have been carried out on sets of single plants deliberately selected to include representatives of a wide range of types in less unequal numbers. By such selection this material may be made eminently suitable for detailed study of the features most closely related to resistance.

Work of this nature has been carried out over three seasons on large numbers of plants, chiefly of U.4 x Sea Island origin, but including also U.4 x Egyptian, and M.U.8 x Sea Island. Results from one set of plants (U.4 x Sea Island) are summarised below and illustrated in figs. 6 and 7, while further data will be found in the Appendix. The results with all the other sets studied were closely similar in the general relationships exhibited, and in the absence of anomalous instances.

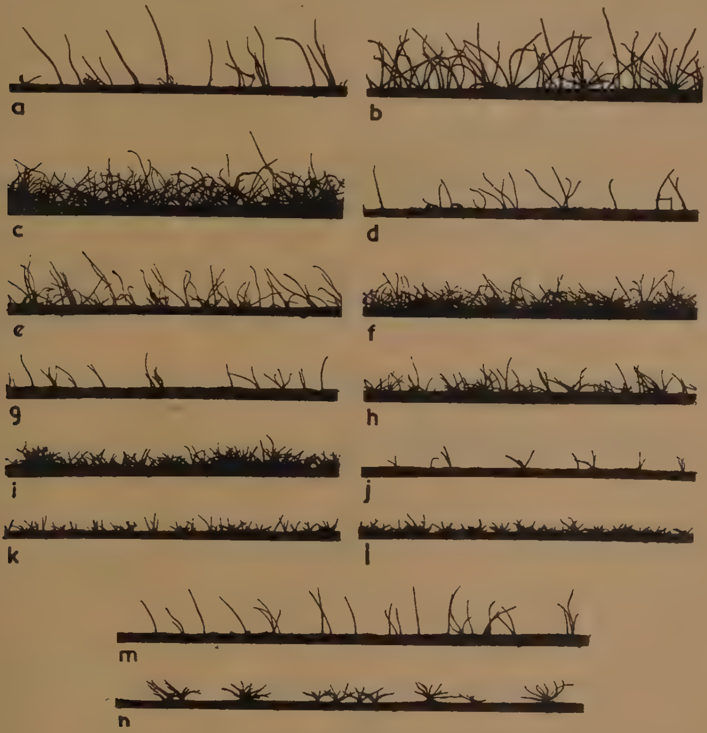

Fig. 7.—Examples of lamina hairiness of hybrid single plants.

Silhouettes (×8) of sections 1·4 mm. wide. Strips *a–l* illustrate range in length and density, except that lower limit of density (zero) is not portrayed. Strips *a–f* are from plants showing marked Jassid resistance, *b* and *c* being virtually immune. Types *g*, *h* and *i* showed some resistance. Types *j*, *k* and *l* were almost as susceptible as glabrous plants. Strips *m* and *n* illustrate extreme range in "style" of hairiness, *m* is closely similar to the U.4 (*G. hirsutum*) and *n* to the Sea Island (*G. barbadense*) parent.

In a field containing a uniformly distributed high proportion of fully susceptible material, a selection was made of 155 plants for counts of nymphs and records of lamina hairs. Four leaves on each of these were sampled for hairs. These gave the following ranges and average values for selected hair characters of the lower surface of the lamina.

Total Density (hairs of all lengths).
30 to 2,750 per sq. cm. Average 757.

Mean Hair Length (all hairs).
0·22 to 0·79 mm. Average 0·44 mm.

Hairs per sq. cm. exceeding specified vertical heights.

Height of 0·3 mm.	0 to 650	Av. 265
Height of 0·4 mm.	0 to 580	Av. 155
Height of 0·5 mm.	0 to 450	Av. 82

The average figures (apart from total density) represent hairiness of about the standard of U.4 cotton or rather better. The range is from about Tidewater standard, to something very definitely better than M.U.8. There was an extremely high range in total density and in the proportion of longer hairs. Thus, on one plant giving a total density of only 510 hairs per sq. cm., 88 per cent. of these exceeded a height of 0·4 mm. At the other extreme, only 0·4 per cent. of the hairs on another plant, with a total density of 2,630, reached this height. These and other features of the plant-to-plant variation, are illustrated in fig. 7.

In spite of the wide range in style of hairiness, it was found that a single count of hairs at a chosen height gave figures highly correlated with infestation. Twice-weekly counts of Jassid, on eight leaves per plant, were continued over a period of 35 days, giving figures equivalent to 88 leaf-counts per plant. The totals per plant ranged from 0 to 371. The latter figure (equivalent to 4,200 nymphs per 1,000 leaves) represents a really heavy infestation (cf. figures in Table I). The geometric mean per plant was 33 (375 per 1,000 leaves) which is roughly what would be expected on *G. hirsutum* plants, with hair figures near the means given above, under similar conditions.

The coefficient of correlation between log. infestation and hairs exceeding 0·4 mm. in height was −·81.* As will be seen from fig. 6, the relationship was very definite indeed, with only comparatively few badly aberrant points. Some of these are explicable by reference to the fact, already pointed out, that for a wide range of hairiness, the choice of a single height for hair counts is a compromise. Fuller analysis of this material showed that counts at heights of 0·5, or even 0·6 mm. are necessary to differentiate satisfactorily between some of the most hairy plants. This point is dealt with more fully in the Appendix.

As in the case of the range of *G. hirsutum* strains of Table I, a multiple regression equation based on counts at heights of 0·3 and 0·5 mm. together gave some improvement over the use of a single count.

Having regard to the nature of the experiment, the discrepancies still remaining might well be attributed solely to errors in determining lamina hairiness, and to chance fluctuations in intensity of infestation. A further investigation was, however, carried out by calculating a predicted value for log. infestation for every plant, by use of multiple regression on lamina hairiness, and studying the deviations from prediction in relation to all other available records on the plants. It was found that the deviations bore some relationship to scores for plant size, fruiting and earliness, but a much more important source of divergencies lay in lack of agreement between hairiness of lamina and that of *midrib*. This point is dealt with in a later section.

In a further attempt to follow up all the wider discrepancies, progenies were raised from the seed of the aberrant plants, and an analysis of similar form carried out on plants in these progenies. In no case was there found to be any indication of an inheritable tendency to deviate from expectation in a particular direction, when the expected value was calculated from knowledge of both lamina and midrib hairiness.

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*With 153 degrees of freedom, the statistical significance of this value is extremely high.

This hybrid material also yielded confirmation of the correspondence between counts of nymphs and degree of Jassid damage to the foliage. Visual assessment and numerical scoring for damage is less easy on single plants of differing appearance, than on strains of *G. hirsutum*, but the plants showed well-marked differences varying from no visible symptoms to bad damage, and could be regarded as covering almost the full range from virtually immune to fully susceptible. The scoring for damage was carried out before the most susceptible plants were too extensively defoliated. It was clear that, in general, the degree of damage was quite closely related to intensity of infestation, and on 155 plants the coefficient of correlation between score for effect (see p. 549) and log. infestation reached the high and extremely significant value of +·80.

(*e*) Plants of Different Ages.

All the foregoing sections deal only with plants sown at the normal period for cotton at Barberton (November-December). Such plants may be expected to have grown far beyond the seedling stage before Jassid infestation commences

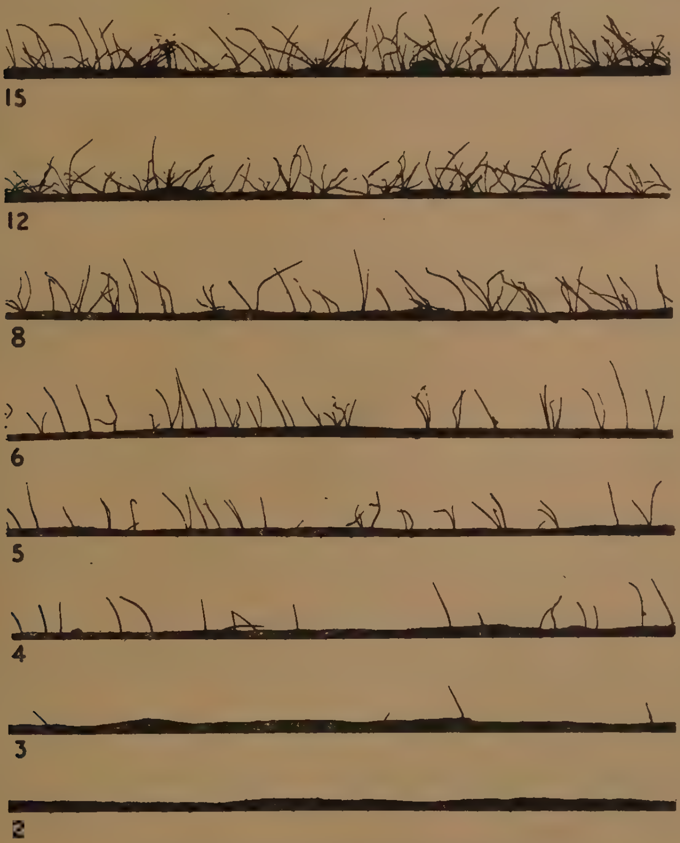

Fig. 8.—Hairiness of successive main-stem leaves.

Silhouettes (×8) of sections 1·4 mm. wide from successive main-stem leaves (numbered from the base upwards) of a plant of strain M.U.8 showing the gradual increase in hairiness from "nil" on leaves 1 and 2 to "full hairiness" at leaf 15.

to be serious. Interesting and enlightening results are provided by plants sown later, when Jassids have already appeared and are breeding in quantity on earlier sown crops.

Thus, in a small experiment in which the strains M.U.8 and Acala were sown in late March, in the vicinity of areas of cotton sown three months earlier, the following figures were obtained for infestation, counting only the *first and second true leaves* of the seedlings, four weeks after germination.

M.U.8 — 2,278 nymphs per 1,000 leaves.
Acala — 2,538 nymphs per 1,000 leaves.

The contrast with the figures in Table I (p. 549) could hardly be more striking: the description of M.U.8 as a highly resistant strain, even in comparison with the very susceptible Acala, does not hold good for the very young plant. The explanation lies in the fact that M.U.8 owes its normal high degree of resistance to the very hairy leaves of the *grown plant*. The first two leaves are essentially *hairless* and thus show *no resistance*.

The following figures for hairiness were obtained, in another experiment, from successive main-stem leaves of young M.U.8 plants on which hair development was unusually good and rapid. (See also fig. 8.)

Main-stem leaves (Counting upwards from the 1st true leaf)	Lamina hairs per sq. cm.		
	Total	Exceeding heights of	
		0·3 mm.	0·5 mm.
1st	0	0	0
2nd	4	2	0
3rd	19	17	10
4th	56	38	24
5th	158	140	96
6th	204	175	122
7th	276	236	164
8th*	338	279	189

*The plants would be roughly 10 weeks old when the 8th leaf is fully expanded.

In view of these figures it is clearly to be expected that very young seedlings of M.U.8 should prove susceptible.

This gradual development of hairiness, as successive leaves are formed, is common to *all* hairy strains of cotton examined at Barberton. The very first leaves on young seedlings are never more than very sparsely hairy, and the full hairiness of which the mature plant is capable does not appear till later.

A lack of resistance, corresponding to this lack of hairiness, has likewise been found in all other strains of which young seedlings have been exposed to infestation. Counts of nymphs have been made on the foliage of young late-sown plants and, at the same time, on leaves produced higher up the stem on older plants growing alongside. By this means direct comparison is possible between leaves of approximately *equal age*, exposed to infestation *during the same period of the season*, but differing in position up the stem and therefore in hairiness.

The figures given below are from a replicated experiment in which the difference in sowing date was such that the third leaf on the younger plants was, on average, comparable in age with the eleventh leaf on the older. From counts on the same single date, the average infestation (geometric means expressed as nymphs per 10 leaves) was as follows :—

	Younger Plant 3rd Leaf	Older Plant 11th Leaf
U.4/5148 ...	50	6
Acala	92	91

It is evident that the third leaf of the U.4 is highly susceptible under conditions in which the eleventh leaf shows considerable resistance, while the very high susceptibility of the non-hairy strain, Acala, is not dependent on the position of the leaf up the stem.

Results from a more elaborate experiment are given in Table II. The comparison between two strains, M.U.8 and Meade, differing very widely in degree of hairiness of the grown plant. Two dates of sowing were employed and an attempt was made to trace the development of hairiness with the growth of the plants of each strain, at each date of sowing, together with changes in infestation from leaf to leaf.

The experiment was sited adjacent to ratoon plants already carrying susceptible foliage when the earlier sowing was made. As may be seen from the figures, infestation appeared on the lowest leaves recorded in the experiment, and reached extremely high levels on the Meade plants. This quickly resulted in severe damage, influencing further infestation and interfering seriously with the determination of hairiness—some leaves were virtually destroyed before samples could be taken. As a result, the extent and accuracy of the comparisons which can be made from the figures are limited, but the general trends exhibited are nevertheless of clear significance.

The figures for the third leaf of the later sowing indicate that before appreciable hairiness had developed on either strain, there was no difference in susceptibility. From this point onwards the progressively increasing differences in hairiness are strikingly reflected in the very rapid divergence of the infestation figures.

TABLE II.

Lamina hairs and Jassid infestation on successive leaves of young plants at two dates of sowing.

Leaf Numbers counting up main-stem (Cotyledons equal "0")	Hairs per sq. cm. of lower surface of lamina at height of ·5 mm.		Jassid nymphs per 100 leaves	
	M.U.8	Meade	M.U.8	Meade
Earlier Sowing				
5–7	47	4	24	188
8–10	194	15	7	249
11–13	215	21	1	535
14–16	459	62	0	675
Later Sowing				
3	(0)	(0)	107	117
4	(16)	(0)	71	331
5–7	30	2	59	663
8–10	192	31	10	944
11–13	259	71	3	529

The hair counts were made on lamina strips taken from the central leaf of each group of three.

The figures given in brackets for the lowest leaves on the later sowing were not obtained from this experiment, but are typical figures for leaves 3 and 4 on these two strains.

The sowing dates were 24th November and 18th December.

There was a natural increase in infestation as the season progressed, as is reflected in the generally higher figures, leaf for leaf, when the later sowing is compared with the earlier. In consequence, within either sowing considered separately, each successive group of leaves up the stem was exposed to heavier infestation than those below it.

In the case of Meade, the infestation figures reflect this increase, but M.U.8, on the contrary, shows a rapid drop. It is clear that the rate of development of hairiness on M.U.8 has been sufficient to reverse the trend of the figures and has led to almost complete resistance at leaves 11 to 13 on both sowings. The development of hairiness on Meade is on a much lower scale and only leaves 11-13 of the later sowing gave any evidence of developing resistance under these very severe conditions.

It is very clear that the time of onset of Jassid infestation and the rate of hair development on the plant must be taken into account in all studies of resistance.

The rate of hair development on a number of strains is illustrated in the Annual Report of the Barberton Station for season 1944–45, page 23 (MacDonald & others, 1946). It had been found that even under comparable and satisfactory growth conditions, varieties differ considerably in the rate at which hairiness increases from leaf to leaf on the young plant, and also in the point up the stem at which this increase ceases. Moreover, changes in density and in length of hairs do not follow parallel courses throughout the season.

These facts, taken in conjunction with the marked effect of conditions of plant growth, to which reference has already been made (p. 542), render it impossible to specify a particular point at which " maximum " hairiness is reached, or to lay down a single standard by which varieties may be compared. It can, however, be said that with *G. hirsutum* strains at Barberton " full " hairiness is to be expected at about the 14th to 18th main-stem leaf and that, beyond this point, there is a tendency for length of hairs to decrease. With some varieties of *G. barbadense* hairiness continues to increase above the 18th leaf.

OTHER PARTS OF THE PLANT.

As already recorded, hairiness of the lower surface of the lamina is commonly associated with hairiness of other parts of the plant. In consequence, evidence of a close relationship between lamina hairiness and resistance to Jassid is not, in itself, proof that lamina hairs are the direct cause of resistance.

It might well be argued, for instance, that resistance was due to hairiness of petiole, but that lamina hair counts served as an efficient estimate of degree of petiole hairiness. Alternatively, it is plausible to regard hairs on lamina, veins, petiole and stem as all likely to contribute to the resistance of the plant, so that lack of hairs on, for instance, the stem must result in lack of full resistance, no matter how hairy the lamina may be. This question became of practical importance at Barberton when hybridisation between strains of *G. hirsutum* and *G. barbadense* gave plants with good lamina hairiness and virtually naked stems.

The possibility that hairs on the leaf veins and petiole might be of more direct importance than lamina hairs is suggested by knowledge that the eggs of *Empoasca* spp. are laid in these parts and rarely, if at all, in the lamina. There is ample evidence on this point, and also that young nymphs can feed and develop when placed on an extremely hairy lamina (Parnell, 1927; Peat, 1928; Verma & Afzal, 1940; Husain & Lal, 1940; Lal & Husain, 1945).

Without some practicable means of removing hairs, the elucidation of these points is dependent on the existence of material for study in which the hairiness on various parts of the plant is not closely related. Given such material, there are still difficulties to be overcome. Accurate comparison demands comparable methods of expressing hairiness numerically, and comparable standards of accuracy in its determination.

Entirely satisfactory means of overcoming these difficulties have not been evolved and, in consequence, the evidence presented in this section lacks finality and, while representing a considerable amount of detailed work, is not put forward as entirely conclusive.

(*a*) Midrib.

A cross section of the midrib reveals a roughly semicircular outline protruding from the lower surface of the lamina, with hairs radiating from the surface (see fig. 9). If, however, the curvature is entirely ignored, a simple and rapid method (closely comparable to that used for lamina) may be adopted for recording hairs exceeding specified vertical heights beyond the surface, measured at right angles to the lamina. Using this method, and taking a standard length from the midrib between petiole and nectary, counts of hairs exceeding a height of 0·5 mm. have been found effective.

Figures were obtained in this way from three leaves of each of 99 of the 155 hybrid single plants discussed above (pp. 556–559). The leaves were taken, however, not from the plants in their first season (1943–44), when the infestation counts were made, but from ratoon growth the following season. In order to obtain a valid comparison between midrib and lamina, these same leaves were utilised to provide new records of lamina hairiness, choosing in this case counts at a height of 0·3 mm. These two sets of figures, when correlated with the 1943–44 records for Jassid infestation, gave the following coefficients :—

Log infestation and lamina hairs – ·69
Log infestation and midrib hairs – ·67

The relationship between lamina hairs and infestation, although lower than that obtained when both records relate to the same foliage, is very definite indeed.* Midrib hairs gave almost the same figure, and it is clear that, with this material, there is nothing to choose between these two measures of hairiness of the leaf.

It must be emphasised, however, that this comparison is not between two perfect quantitative evaluations of hairiness, but between two chosen measures, not necessarily comparable in accuracy of determination, and in neither case an exact numerical representation of the observable characteristics of the hairy cover. In respect of size of sample, midrib is favoured, but the accuracy of determination, on the sample, is probably higher in the case of lamina.

The relative magnitude of the two coefficients may be varied quite considerably by the arbitrary exclusion of a few plants from the calculation, and the comparison is obviously very dependent on the choice of material. With other data studied in this way (from both hybrid single plants and *G. hirsutum* plants and strains) somewhat inconsistent results have been obtained, with midrib often giving rather the higher coefficient.

There is a marked positive correlation between the lamina and midrib hairiness, the coefficient being +·72 in the above example, and +·67 in the case of a set of U.4 (*G. hirsutum*) single plants, so that when *either* is correlated with infestation, it follows inevitably that the other must be also.

To overcome this limitation as far as possible, material has been studied in which this relationship, between hairiness of lamina and of midrib, was considerably reduced by deliberate selection. Seed was collected from plants, in hybrid material, showing wide departures from the " average " relationship. Numerous single plants raised from this seed were then subjected to heavy infestation and sampled for lamina and midrib hairs.

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*At the very stringent standard of P = ·001 any value over ± ·34 is significant. The value of — ·69 given above compares with the value — ·76 obtained when using lamina hair counts made in 1943–44, the season in which the Jassid counts were made.

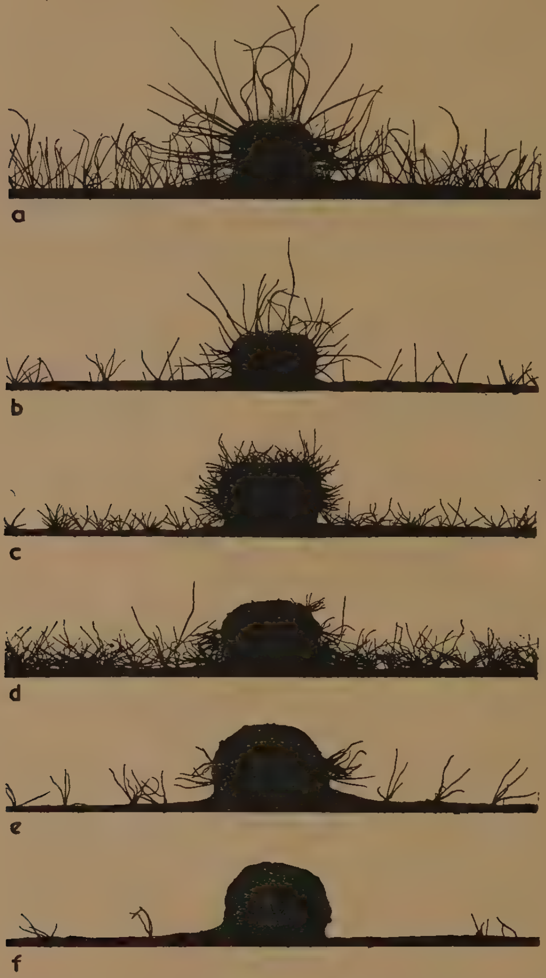

Fig. 9.—Hairiness of midrib.—Silhouettes (×8) of cross sections, approx. 1·4 mm. thick, taken near base of midrib. (*a*) and (*b*) show *hirsutum* style, although (*a*) is in fact from a hybrid plant. (*b*) is U.4/5143. (*c*) also from a hybrid, shows high density but no increase in length over lamina hairs. (*d*), (*e*) and (*f*) are *G. barbadense* types, (*d*) being the Jassid resistant Woolly NT 2, and (*f*) Sea Island.

The result was a set of 104 plants in which the correlation between midrib and lamina was reduced to + ·49. For this analysis the midrib hairs were recorded by a more refined (and much more tedious) method using cross sections. The " heights " were measured radially from the curved outline over an arc extending to within 0·3 mm. of the lamina on either side, and the counts expressed as hairs per unit area of surface examined.

The distribution of the 104 plants as regards degree of hairiness of lamina and midrib is set out in Table III. It will be seen that, although far from an ideal cover of all combinations, the spread is sufficient to enable some dissection of the separate effects of one character, with the other held within limits at a number of levels.

The Table also gives the average Jassid infestation on the plants of each combination of hair measures, and reveals that increasing resistance follows increase in *either* measure of hairs, at various levels of the other.

Numerous partial correlation and regression coefficients have been calculated from these data on a plant by plant basis, using various sub-divisions and, in spite of the imperfections of the data, these leave little doubt that hairs on lamina and on midrib *each* play a significant part in conferring resistance.* As is clear, however, from inspection of the figures in the table, the effect of changes in level of one character is appreciably dependent on the level of the other. Thus, when lamina hairiness is high, changes in degree of midrib hairiness appear to have little effect, as a high degree of lamina hairiness alone is sufficient to reduce infestation to a low figure. Similarly, where midrib hairiness is very high, only the highest degree of lamina hairiness affects the resistance.

TABLE III.

Jassid infestation on groups of hybrid plants differing in degree of hairiness of lamina and midrib.

Midrib hairs		Lamina hairs				
		Very low (0–20)	Low (21–50)	Moderate (51–100)	High (101–150)	Very high (over 150)
Very Low (0–24)	Plants	9	8	6	2	—
	L & M	9 9	35 12	68 10	117 11	
	Nymphs	481	242	128	47	
Low (25–49)	Plants	2	9	9	—	—
	L & M	13 45	29 36	67 39		
	Nymphs	296	243	108		
Moderate (50–74)	Plants	—	5	10	1	2
	L & M		43 59	73 65	104 54	270 65
	Nymphs		146	120	140	14
High (75–99)	Plants	—	7	5	3	4
	L & M		36 84	78 87	123 89	195 89
	Nymphs		67	41	22	2
Very High (over 100)	Plants	—	3	11	3	5
	L & M		33 117	69 123	115 128	310 149
	Nymphs		53	55	56	2

Plants=Number of plants in group on which all averages are based.

L=Lamina hairs per 100 sq. mm. of lower surface, reaching a height of 0·5 mm.
M=Midrib hairs per 20 sq. mm. at a radial distance of 0·6 mm.
Nymphs=Geometric means of counts of Jassid nymphs, expressed as nymphs per 100 leaves.

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*Some statistics are given in the Appendix.

Unfortunately, as the Table shows, plants combining a very high degree of hairiness in one character with a very low degree of hairiness in the other did not occur in this material. The gap can, however, be partially filled by the Woolly N.T.2 plants referred to earlier (p. 555), which were grown in the same season in a nearby field and were also heavily exposed to infestation. Eight plants of this strain gave mean figures for lamina and midrib hairs, recorded as for the plants in Table III, of 283 and 15 respectively, and would thus come into the top right hand corner of the Table. The infestation per 100 leaves was 13·5, a figure which fits in very well indeed with those presented in the Table. The appearance of a typical midrib of this type, in cross-section, is illustrated in fig. 9.

(*b*) Petiole and Stem.

Reference has already been made to the hairiness of petiole and stem in relation to that of leaf. Comparative figures for a range of types are given in the annual report of the Station for season 1942–43 (Macdonald and others, 1944). In general within the species *G. hirsutum* there is a close relationship. In the *G. barbadense* types examined (other than Tanguis) hairy leaves occur in conjunction with naked stems, while in hybrids between the two species hairiness of lamina may be associated with either hairy or non-hairy stems.

When stem differs conspicuously from lamina the petiole often tends to be intermediate between the two in degree of hairiness, but very substantially hairy leaves (approaching the standard of the *G. hirsutum* strain, M.U.8) have been found with both stems and petiole virtually naked.

Such plants show high resistance to Jassid, and it is at once clear, therefore, that stem and petiole hairs are not essential; but it remains of importance to determine whether they confer added resistance. Hairy stems and petioles have not been found in conjunction with glabrous leaves, so that the status of this combination in relation to Jassid cannot be investigated.

No rapid method of obtaining reliable measurements of stem and petiole hairs has been developed. The problems of sampling for microscopic examination are formidable since the hairs are frequently most unevenly distributed and, in the case of stem at least, are undoubtedly deciduous. Adequate sampling before hairs are shed involves mutilation of the plant and premature termination of infestation records.

For these reasons studies have been confined to eye-judgment of hairiness, the limitations of which have already been referred to. Even with the most difficult material, however, the separation of contrasting classes such as " substantially hairy " (hairs both numerous and long) and " non-hairy " (stems virtually naked) can be made with ease and confidence, the more dubious intermediate degrees being omitted from consideration.

By analogy with the results obtained with lamina, a comparison between groups such as this should prove sufficient to reveal the presence of any appreciable effect. The result of a comparison made in this way with hybrid single plants in 1942-43 was as follows :—

Nineteen plants with non-hairy stems.

Nymphs per 100 leaves	31
Score for Jassid effect	2·1

Sixty-three plants with substantially hairy stems.

Nymphs per 100 leaves	21
Score for Jassid effect	1·6

The lamina hairiness of all the plants included was classed by eye-judgment as " moderate ", and the figures reflect this in showing a very substantial degree of resistance for *both* groups, but there is a definite indication that hairy stems have resulted in added resistance.

A very similar result was given by 1943-44 hybrid material, using hair counts to ensure equality of *lamina* hairiness for the two groups of stems, and including a group of fully susceptible plants to indicate the degree of exposure to infestation.

	Nymphs per 100 leaves	Score for Jassid effect
Ten plants with both leaves and stems non-hairy	301	6·8
Ten plants with moderate lamina hairiness, stem hairs " Nil " or " Trace."	28	0·8
Eleven plants with moderate lamina hairiness, stem hairs numerous and long	17	0·6

Further investigation in season 1944-45, again using hybrid material of the same origin, suggests however that both these results may have been due entirely to inequality of *midrib* hairs between the stem groups. Only lamina hairs were held comparable, and in this material the association between stem and midrib is appreciably greater than between stem and lamina.

With both lamina *and midrib* hairiness held approximately uniform, the range in stem hairiness is much reduced, but comparisons may still be made if the stem grouping is modified.

The following figures were obtained using the stem descriptions available for the plants of Table III (p. 565), taking three levels of leaf hairiness :—

	Nymphs per 100 leaves
Both lamina and midrib hairiness " Very Low "	
Stems naked (5 plants) ...	464
Stems with some hairs (4 plants) ...	501
Both lamina and midrib hairiness " Low "	
Stem hairs " Nil " or " Trace " (5 plants)...	237
Stem hairiness appreciable (4 plants)...	249
Both lamina and midrib hairiness " Moderate "	
Stem hairiness low (4 plants)	120
Stem hairiness high (4 plants)	97

There is here no indication that stem hairs have any appreciable influence, though the numbers of plants available are too small to put the matter beyond doubt.

General Discussion and Practical Implications.

The data presented in the foregoing sections provide, in the opinion of the authors, conclusive proof of an extremely close relationship between " hairiness " and " Jassid-resistance " with *Empoasca facialis* at Barberton. Degree of hairiness, measured in an appropriate manner, has proved an unfailing guide to resistance in all the material studied. It is further considered that the evidence points emphatically to a simple relationship of direct cause and effect, and not to a genetic linkage between hairiness and some other factor conferring resistance.

The following points support this view :—

(*a*) The relationship is consistent over a very wide range of material of diverse origins. Substantially hairy foliage invariably shows resistance and non-hairy plants are without exception susceptible.

(*b*) The wide and continuous variation in degree and style of hairiness points to the existence of a considerable number of genes controlling the development of hairs. Yet, in all cases, appreciable increases in degree of hairiness are accompanied by corresponding increases in resistance, irrespective of the origin and genetic diversity of the material. The relationship holds for plant-to-plant comparisons within any one strain, irrespective of the general style of hairiness exhibited by that strain. It likewise holds in hybrid material in which the style as well as the degree of hairiness varies widely.

(*c*) The non-hairy foliage of seedlings is highly susceptible to Jassid, irrespective of the status of the mature plant, and when hairiness develops with the growth of the plant, resistance develops concurrently and at a corresponding rate.

(*d*) There is evidence that the decrease in effective hairiness which accompanies the expansion of the growing leaf is associated with a decrease in resistance.

The last two points indicate that, *with the genetic composition of the material constant,* changes in hairiness cause changes in resistance.

It is not maintained that hairiness is the sole factor concerned in controlling either infestation or susceptibility to damage. Differences in growth and cropping undoubtedly exert some small influence on both the attractiveness of the foliage to Jassid, and the reaction of the plant to a given degree of infestation. Heavy fruiting, and consequent reduced vegetative growth, appears to render the plant more rapidly susceptible to damage, although higher counts of infestation are given by less fruitful plants with continued strong vegetative growth.

Under any but the most abnormal conditions, however, all such factors are clearly of very minor importance, relative to hairiness. In the 1943-44 hybrid single plant material, selected solely to cover a wide range in hairiness, the variations in plant size, habit and fruiting were extreme; leaf size, shape and texture also varied widely and the plants were scattered over more than an acre of land. Thus, various causes were liable to limit the accuracy of determinations of both hairiness and resistance: yet almost two-thirds (65·9 per cent.) of the variability in infestation can be accounted for in terms of hairiness, using only a single measure of lamina hairs.

When the comparison is between *G. hirsutum* strains, in replicated plots, resistance follows lamina hairiness so closely that it is clear that no other character (unless closely linked with hairiness of lamina) can be of any real importance. Thus, in the case of the 1944-45 experiment, the regression on lamina hairs exceeding a height of 0·3 mm. accounts for 97·4 per cent. of the total variability in Jassid infestation between the ten strains, measured by the sums of squares of deviations using a logarithmic transformation. The strains included were by no means closely alike in growth, fruiting and other plant characters, and differed appreciably in the texture of their leaves.

Mechanism of Resistance.

The direct and extremely marked effect of hairiness in conferring resistance is now well established, but the mechanism of this resistance is by no means understood. No attempt has been made to investigate this problem and the remarks that follow are based on general experience of the pest.

It may be assumed that nymphs do not move freely from plant to plant and cannot, therefore, choose their host. This choice must be made by the adult female and, in general, a heavy infestation on any plant must indicate heavy oviposition on that plant. Whether the reverse is true is not altogether certain, but such evidence as there is supports that assumption.

Verma and Afzal (1940), working with *E. devastans*, showed that fewer eggs were laid on resistant types of cotton, but that the percentage of hatching and the survival rate of nymphs, in cages, were not affected by " resistance ". They do not state whether the *rate of development* was affected, but even supposing this were reduced by hairiness, through interference with feeding or by other means, thus retarding the *build-up* during the season, this would not affect the point at issue. Hairy plants exposed to a heavy adult population, bred up on adjacent susceptible types, still show the reduced nymphal infestation associated with " resistance ".

Though the survival of nymphs in captivity is not affected by hairiness of the leaf, they might be more vulnerable to predators in the open, due to the hairs hindering their activity. Such action would reduce the survival rate and result directly in reduced infestation. It seems unlikely, however, that the steady additive effect of increasing hairiness, over a very wide range in degree and style, is explicable on this basis. Moreover, where lamina hairiness is low, midrib hairs alone may confer an additional high degree of resistance, although it appears most unlikely that they could affect the survival rate in the same manner as lamina hairs. Thus, there appears to be good reason for supposing the " resistance " effect of hairiness to be mainly a matter of *reduced oviposition* by the adult female.

The eggs are laid in the midrib and larger veins of the leaf and in the petiole, more rarely in the young stem. In hairy *hirsutum* cottons, all these parts are hairy ; yet the female does lay eggs in them, even if to a limited extent. It follows that hairiness does not make oviposition impossible, but merely discourages it. A point of interest is that such discouragement may be affected by a hairy lamina only, where stem and petiole are essentially glabrous and the greater part of the midrib is also devoid of hairs. Thus, the preference of the gravid female for less hairy plants is not governed essentially by the condition of the actual points of oviposition.

No attempt has been made to investigate the general reaction of the adult to hairiness of the leaf. There may be a simple distaste for hairiness and a preference for less hairy plants quite unconnected with egg-laying. Whatever the reason for the " preference " that does exist, it is most remarkable in that it is so finely graded. Judged by the results of all experiments, the Jassid can, and almost unfailingly does, detect and react appropriately to all distinguishable degrees of hairiness measured by the methods described in this paper. It is the more remarkable that both extremes in the range of hairiness are covered by this graded preference : thus the very few hairs of Coker Wilds render it rather less susceptible than Acala, which has even less, whilst at the other end of the range the very hairy strain A.2106, although very highly resistant, is less so than the still more hairy M.U.8.

Breeding for Jassid Resistance.

The practical implications of the results obtained are not, of course, dependent on the exact mechanism of resistance. It is abundantly clear that breeding for hairiness is a thoroughly satisfactory method of obtaining Jassid resistance.

As a result of the information now available, the requirements for high resistance can be stated with confidence. Length of hairs is of prime importance and the longer they are, the better. Provided length is maintained, the higher the density, the greater the assurance of high resistance. As explained in the introductory discussion of hairiness, it has been found satisfactory to consider length in terms of vertical height and to bring in the density factor by using counts of hairs per unit area exceeding a specified height. There is no sharply defined critical point separating effective length from non-effective but, for practical purposes, where a substantial degree of resistance is needed, it seems certain that hairs of less than a vertical height of 0·3 mm. can be entirely disregarded. Above this height the experimental results show clearly that the standard required depends on the level of infestation to which the crop is exposed.

Nevertheless, even where Jassid incidence is normally such that a very moderate degree of hairiness confers sufficient resistance, a higher standard provides a safeguard against the effects of poor growing conditions on hair development, and will also result in resistance developing earlier in the life of the young plant.

For the conditions experienced at Barberton, quite definite standards for lamina hairiness can be set. To ensure high resistance under the most extreme conditions likely to be met with locally, the standard for height should be 0·5 mm. and the density at this height should approach 150 hairs per sq. cm. This is approximately the standard of strain A.2106.

A less stringent standard, quite satisfactory under all normal conditions, is represented by the average figures for local U.4 cotton. These are in the neighbourhood of 60 hairs per sq. cm. at a height of 0·5 mm. with about 200 exceeding a height of 0·3 mm. Counts at 0·5 mm. of less than 60 must be regarded as inadequate, even with far greater densities at lower heights.

In handling material in which midrib hairiness does not follow that of lamina, however, it may prove necessary to include midrib hairs as a factor in plant selection, or to set an even higher standard for lamina.

The results given in this paper suggest that hairiness of petiole and stem may safely be ignored, but it should not be overlooked that, in *G. hirsutum* material, hairiness of stem has been found a satisfactory guide to hairiness of leaf, and is more easily judged in the field. It has also been found, in breeding work with interspecific hybrids, that progenies breeding true for lamina hairiness are more easily obtained from selections carrying good stem hairs.

In general breeding practice, at least with *G. hirsutum,* it should not prove necessary to do more than select " hairy plants " by eye, provided only that sufficient attention is given to *length* of hairs.

In all cases of suspicion that the relationship between hairiness and resistance has " broken down ", the actual foliage concerned should be carefully examined. A strain that is normally described as " hairy " is not resistant if it is not carrying hairs, e.g., the young plants of M.U.8, to which reference has been made before.

It may be noted here that, where types of cotton differing widely in resistance are planted in large blocks, the build-up of Jassid population inside the blocks varies enormously. Thus a whole field of M.U.8 or A.2106 may be practically free from both adults and nymphs, whereas a patch of Coker Wilds not far away may be shimmering with adults on the wing. The experimental material dealt with in this paper has always been exposed to a uniform adult population, bred up on susceptible types mixed in for the purpose, so that differential build-up has not affected the results. It follows that a resistant type grown by itself in general cultivation exposed only to its own build-up of population, will be more effectively resistant than these results indicate.

Summary.

A wide range of material has been studied in an investigation of the relationship between " resistance " to attack by *Empoasca facialis* and " hairiness " of the cotton plant.

A continuous range has been found between full susceptibility and very high resistance.

In general relative susceptibility, as gauged by visual assessment of symptoms, has been found to correspond closely with relative infestation, determined by counts of nymphs.

With attention confined to grown plants, the *relative* susceptibility of varieties has been found substantially constant under varying levels of exposure to infestation.

Hairiness of the cotton plant has been found to show an extremely wide range in degree, style and distribution. A method has been devised for expressing hairiness numerically, taking into account both length and density of hairs.

A very close and consistent relationship has been found between degree of hairiness of the under surface of the leaf, and degree of resistance to Jassid. Without exception, all thoroughly hairy types have been found highly resistant, and all non-hairy types fully susceptible. Intermediate degrees of hairiness are associated with intermediate degrees of resistance.

The relationship has been found to hold good between varieties and between plants within varieties, of the species *G. hirsutum*; also between plants of *G. barbadense*, and in segregating progenies of hybrids between these two species.

The hairiness of resistant strains of cotton has been found to develop gradually with the growth of the plant; the first few leaves on the seedlings being virtually non-hairy. This lack of hairiness in the early stages of growth is associated with a lack of resistance. Hairiness and resistance to Jassid develop concurrently.

The conclusion is reached that hairiness of the leaf *confers* resistance to Jassid and that degree of leaf hairiness, measured in an appropriate manner, is a thoroughly reliable guide to degree of resistance.

Length of hairs is shown to be of prime importance, and high densities without adequate length are ineffective.

The relative importance of hairs on lamina and midrib has not been conclusively determined. Both have an influence on resistance but a high degree of midrib hairiness is not essential if the lamina is hairy.

Hairs on stem and petiole are shown to be of very little direct importance.

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Mr. C. E. Booth was responsible for the whole of the records of infestation and did much of the counting of hairs on many thousands of photographs. Mrs. A. N. de Maine and two visiting scientists, Snr. F. Oliveira Santos and Eng. L. Salazar d'Eca, also assisted in this task.

The technique of obtaining a photographic record of hairiness was developed from preliminary work of Mr. W. L. Fielding.

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Mr. Parnell wishes it to be recorded that the real body of the work described in this paper, including the photographic estimation of hairiness and the very voluminous computations, was carried out entirely by his co-authors.

References.

AFZAL, M. (1941). Present position as regards breeding for Jassid resistance in cotton.—Publ. Indian cent. Cott. Comm., 2nd Conf. sci. Res. Wkrs. India.

—— & MANZOOR ABBAS. (1944). Cotton Jassid (*E. devastans* Dist.) in the Punjab. V. A. note on the characters of the plant associated with Jassid resistance.—Indian J. Ent., **5**, pp. 41-51.

HUSAIN, M. A. (1938). The cotton Jassid.—Publ. Indian cent. Cott. Comm., 1st Conf. sci. Res. Wkrs. India.

—— & LAL, K. B. (1940). The bionomics of *Empoasca devastans* Distant on some varieties of cotton in the Punjab.—Indian J. Ent., **2**, pp. 123-136.

KING, H. E. & RUSTON, D. F. (1945). Progr. Rep. Exp. Stas Emp. Cott. Gr. Corp., 1943-44, p. 36.

LAL, K. B. (1941). Cotton Jassids and their control.—Indian Fmg., **11**, p. 465.

—— & HUSAIN, M. A. (1945). Hairiness of cotton leaves and anti-Jassid resistance.—Curr. Sci., **14**, pp. 153-154.

MACDONALD, D., FIELDING, W. L., RUSTON, D. F. & KING, H. E. (1946). Progr. Rep. Exp. Stas Emp. Cott. Gr. Corp., 1944-45, pp. 14-28.

——, RUSTON, D. F. & KING, H. E. (1944). Progr. Rep. Exp. Stas Emp. Cott. Gr. Corp., 1942-43, pp. 18-36.

PARNELL, F. R. (1927). Rep. Exp. Stas Emp. Cott. Gr. Corp., 1925-26 pp. 19-44.

——. (1928). Rep. Exp. Stas Emp. Cott. Gr. Corp., 1926-27, pp. 52-62.

——. (1935). Emp. Cott. Gr. Rev., **12**, pp. 177-182.

—— & MACDONALD, D. (1942). Progr. Rep. Exp. Stas Emp. Cott. Gr. Corp., 1941-42, p. 23.

PEAT, J. E. (1928). Rep. Exp. Stas Emp. Cott. Gr. Corp., 1926-27, p. 119.

VERMA, P. M. & AFZAL, M. (1940). Studies on the cotton Jassid (*Empoasca devastans* Distant) in the Punjab. I. Varietal susceptibility and development of the pest on different varieties of cotton.—Indian J. agric. Sci., **10**, pp. 911-926.

WILLIAMS, C. B. (1937). The use of logarithms in the interpretation of certain entomological problems.—Ann. appl. Biol., **24**, pp. 404-414.

APPENDIX.

Note on Statistical Treatment of Data.

Transformations.

In general throughout the experiments described in this paper, the numerical results have been so striking that elaborate statistical treatment has not been necessary to bring out the main features. Reference has been made at various points, however, to the use of a logarithmic transformation and *geometric* means of infestation data.

The need for some transformation lies in the nature of changes in insect populations (Williams, 1937), and the crude figures for counts of nymphs exhibit features clearly pointing to the need for logarithmic treatment.

Thus, in the 1943-44 experiment with strains of *G. hirsutum* (see Table I, p. 549) the extreme range in infestation per plot (total nymphs on 180 leaves) was from 0 to 1,041.

Variations between replicates of the same variety were closely dependent on the general level of infestation on that variety, and hence differed very widely indeed over the whole experiment. The figures below illustrate this, and indicate the advantage obtained by using a transformation of the form $y = \text{Log.}\ (x+1)$, where x is the observed infestation per plot.

Variety	Observed Infestation		Transformed Values	
	Mean of 4 plots	Plot to Plot Range	Mean of 4 Plots	Plot to Plot Range
M.U.8	0·75	2	·20	·48
A.2106	7·50	5	·91	·30
BP.50/3	15·0	15	1·18	·40
P.43 F	20·5	17	1·32	·32
BP.52 H	21·3	22	1·30	·54
U.4/5143	20·8	21	1·30	·47
C.9363	42·8	61	1·59	·61
U.4/8161	42·0	20	1·63	·21
BP.52 LH	99·5	78	1·98	·35
Meade	293	307	2·43	·51
Coker	572	390	2·74	·34
Tidewater	575	271	2·75	·21
Acala	715	505	2·84	·29

The plot to plot range shown by the transformed values is clearly quite independent of the mean and a common standard error may therefore be calculated by analysis of variance.

Omitting M.U.8, the most extreme varietal comparison is represented by the difference between 0·91 and 2·84 (against 7·5 and 715·0 for the crude values), while the difference between A.2106 and BP.50/3 becomes comparable in magnitude to that between Meade and Coker. With a standard error of 0·069, both these two latter comparisons indicate "significant" varietal differences.

The further great advantage in the use of logarithms, that of rendering the relationship between infestation and hairiness approximately linear, is very clearly brought out in fig. 3. Without transformation there is always an obvious departure from a straight line relationship.

Alternative Measures of Hairiness.

The choice between alternative measures of hairiness has been largely based on consideration of the *degree of correlation* shown with infestation.

The basis of choice, and the relative merits of a number of alternative counts of hairs (from the point of view of predicting resistance) are well illustrated in fig. 10.

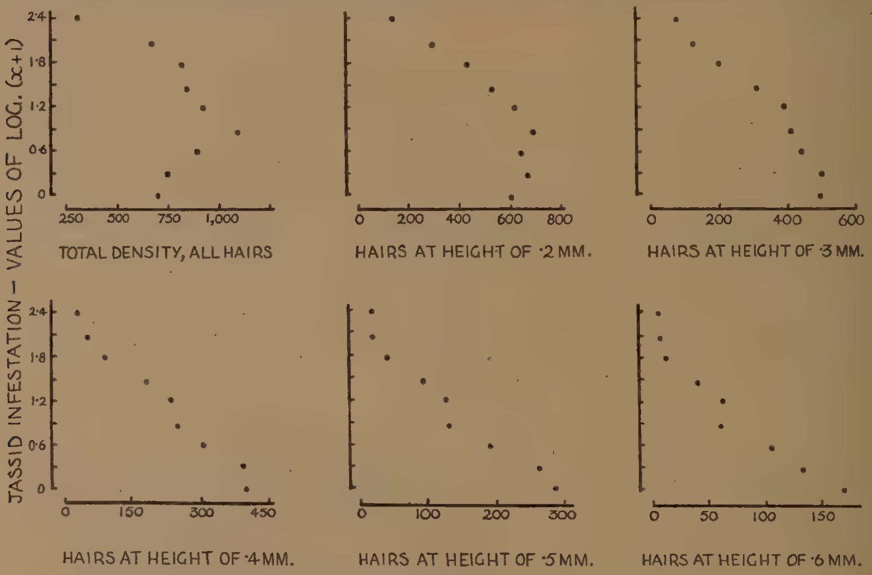
The data for these diagrams were obtained by grouping 155 hybrid single plants according to degree of infestation, on the logarithmic basis, to give nine classes almost equally spaced between full susceptibility and very high resistance. The nine points so obtained are plotted against the group means for the various hair counts.

It will be seen that counts at a vertical height of 0·2 mm. give a close and linear relationship for the more susceptible groups only, while to distinguish between the most resistant, a height of 0·5 mm. is the minimum. Counts at heights of 0·4 or 0·5 mm. are reasonably efficient over a wide range in this material.

The lack of agreement between total density and infestation is perhaps brought out still more clearly by the figures given below. Amongst the more resistant groups there is a negative correlation between total density and mean hair length, with the result that the figures for infestation and density increase together from classes 1 to 4.

Infestation (nymphs per 88 leaves)	Total Density Hairs per sq. cm.	Mean Hair Length mm.
0	692	·63
1·0	730	·58
3·1	888	·53
6·5	1,083	·49
15·6	920	·50
28·1	833	·46
61·7	814	·38
118·4	668	·35
259·6	295	·38

It must be emphasised that the grouping of plants, here resorted to for simplicity in presentation, was not used in the original investigations. All deductions are based on analyses of the data on a single plant basis.



TOTAL DENSITY, ALL HAIRS

HAIRS AT HEIGHT OF ·2 MM.

HAIRS AT HEIGHT OF ·3 MM.

HAIRS AT HEIGHT OF ·4 MM.

HAIRS AT HEIGHT OF ·5 MM.

HAIRS AT HEIGHT OF ·6 MM.

Fig. 10.—Infestation and lamina hair counts on groups of hybrid plants.

Midrib versus Lamina.

Detailed statistical analysis, using methods of partial correlation and regression, was carried out on much material in attempts to determine the relative importance of midrib and lamina hairiness. The following statistics relating to the data discussed above (p. 563) illustrates the nature of the results obtained.

Making use of the figures for 104 plants, without grouping in any way, the coefficients of correlation were as follows :—

	Total Correlation	Partial Correlation
Log. Infestation and Lamina Hairs ...	— ·64	— ·50
Log. Infestation and Midrib Hairs ...	— ·61	— ·44

Both the partial coefficients are significant at P = 0·001 and indicate that infestation is correlated with variations in hairiness on either lamina or midrib separately, with variation on the other eliminated.

An important point, not at once evident in the figures as presented in Table III, is the much higher mean level and much greater range shown by midrib hairs. Both lamina and midrib hairiness falls to zero on some plants, but the upper limit of variation, expressed in terms of the same unit area in each case, is very much higher for midrib. The mean level of hairiness of midrib on 104 plants was over four times as great as that of lamina. A difference of this same order is common in *G. hirsutum* material; the midrib carries a greater density of hairs of greater length. In Table III this is allowed for by using a smaller unit area in expressing midrib hairiness.

As a result, the partial regression coefficients, which measure the change in infestation associated with unit change in hairiness, are not directly comparable. Figures calculated for the 104 plants, on a basis of hairs per sq. mm. for both, were as follows :—

Partial regression of log. infestation on Lamina Hairs	−0·290 ± 0·050
Partial regression of log. infestation on Midrib Hairs	−0·086 ± 0·017

On this basis, changes in lamina hairiness appear far more influential than changes on midrib. In contrast, if the regressions are expressed in terms of the standard deviations of the variables (i.e., if the " Beta coefficients " are calculated), the values obtained are −0·45 and −0·39. Thus, if the range of variation is taken into account, the influence of midrib is of the same order as that of lamina.

The above statistics represent average effects over a wide range. Further calculations show that the effect of one variable is not constant at varying levels of the other. Thus taking separately the groups of plants represented by the five horizontal lines of Table III, the values of the partial regressions of log. infestation on lamina hairs are as follows :—

Midrib hairiness	" Very Low "	(25 plants)	— ·80 ± ·20
,, ,,	" Low "	(20 ,,)	— ·78 ± ·38
,, ,,	" Moderate "	(18 ,,)	— ·33 ± ·08
,, ,,	" High "	(19 ,,)	— ·41 ± ·12
,, ,,	" Very High "	(22 ,,)	— ·20 ± ·06

The effect of unit change in lamina hairiness decreases notably with increasing midrib hairiness, although still significant for the highest class of midrib.

The corresponding calculations using the columns of the Table are based on a less satisfactory distribution of plants, but provide the following figures for partial regression of log. infestation on midrib hairs :—

Lamina Hairiness	" Low "	(32 plants)	— ·137 ± ·033
,, ,,	" Moderate "	(41 ,,)	— ·060 ± ·027
,, ,,	" High and Very High "	(20 ,,)	— ·024 ± ·026

The effect of changing midrib hairiness decreases as lamina hairiness is increased and is not significant on plants carrying really good lamina hairs.

GENERAL INDEX.

A.

A19 (see DDT).
Abyssinia, new *Tribolium* from, **43.**
Achras sapota, *Icerya* on, in India, **536.**
Achrysocharella ritchiei, parasite of *Leucoptera* in E. Africa, **406, 408, 410**; bionomics of, **413.**
aconitus, *Anopheles*.
Acrocercops bifasciata, parasite of, in E. Africa, **413.**
acuta, *Plusia*.
acuticollis, *Rhytinota*.
adenensis, *Anopheles* (*Myzomyia*) *culicifacies*.
adersi, *Aëdes* (*Diceromyia*).
Adlerius, a subgenus of *Phlebotomus*, **98.**
Aëdes adersi, in Kenya, **490.**
Aëdes aegypti, habits of, in forests in Kenya, **489**; in Socotra, **222**; in Sudan, **143, 152**; breeding in plant axils in Uganda, **195–210**; toxicity of limewash containing DDT and "Gammexane" to, **179–183**; reactions of, to stimuli, **387–397.**
Aëdes africanus, habits of, in forests in Kenya, **490.**
Aëdes arabiensis, in Sudan, **143, 152.**
Aëdes argenteus, breeding habits of, **201, 206.**
Aëdes circumluteolus, in Sudan, **143.**
Aëdes deboeri, habits of, in forests in Kenya, **489.**
Aëdes dentatus, habits of, in forests in Kenya, **489.**
Aëdes granti, male and larva of, in Socotra, **226.**
Aëdes haworthi, habits of, in forests in Kenya, **490.**
Aëdes ingrami, habits of, in forests in Kenya, **489.**
Aëdes keniensis, habits of, in forests in Kenya, **490.**
Aëdes kochi var. *samoana*, **205.**
Aëdes marshalli, habits of, in forests in Kenya, **490.**
Aëdes metallicus, **206**; in Sudan, **143, 152.**
Aëdes ochraceus, in Sudan, **143, 152.**
Aëdes pulchrithorax, habits of, in forests in Kenya, **489.**
Aëdes simpsoni, breeding in plant axils in Uganda, **185–212.**
Aëdes unilineatus, in Sudan, **143, 152.**
Aëdes variegatus, breeding habits of, **201.**
Aëdes vittatus, characters of, in Socotra, **227.**
Aëdimorphus (see *Aëdes*).
Aëdomyia africana, in Sudan, **143, 146.**
aegypti, *Aëdes* (*Stegomyia*).
aegyptiaca, *Icerya*.
aethiops, *Stethorus*.
afra, *Atoposoma variegatum*.
Africa, East, annotated list of insects associated with groundnuts in, **231–236**; bionomics of mosquitos in, **185–212, 453–465, 489–490**; isolated generations of *Glossina* in, **441–451**; bionomics of *Leucoptera coffeella* in, **399–416.**
Africa, French West, new *Tribolium* from, **41.**
Africa, South, Coccinellids in, **307–308**; hairiness of cotton and Jassid resistance in, **539–575**; new *Tribolium* from, **46.**
Africa, West, traps for *Glossina* in, **491–528**; *G. tachinoides* and trypanosomiasis in, **529.**
africana, *Aëdomyia*.
africanus, *Aëdes*; *Closterocerus*; *Hallirhotius*; *Taeniorhynchus* (*Mansonioides*).
Ageniaspis, parasite of *Leucoptera* in E. Africa, **406, 410.**
Agrostis canina, relation of, to tick ecology in Wales, **331–337.**
aitkeni, *Anopheles*.
akamushi, *Trombicula*.
Alcidodes (*Alcides*) *dentipes*, on groundnuts in Tanganyika, **235.**
alcine, *Tribolium*.
alfreddugesi, *Eutrombicula*.
Allodontermes morogorensis, attacking groundnuts in Tanganyika, **232.**
Alphitobius, **25, 26.**
alutacea, *Physonota*.
amabilis, *Rodolia*.
Amblyomma variegatum, experiments with "Gammexane" and DDT against, **269–276, 424, 433.**
America, *Stethorus* species in, **297.**
amictus, *Anopheles*.
Ananas comosus (see Pineapple).

Anaphe moloneyi, *Tribolium anaphe* in nests of, in N. Nigeria, **35.**
anaphe, *Tribolium*.
Anaphlebotomus, a new subgenus of *Phlebotomus*, **99.**
Andaman Islands, *Rodolia andamanica* in, **536.**
andamanica, *Rodolia*.
Andropogon, Coccinellid predacious on mite on, in India, **299.**
annularis, *Anopheles*.
annulipes, *Anopheles*.
Anopheles aconitus, bionomics of, in Burma, **243–262**; systematic notes on, **262–265.**
Anopheles aitkeni, bionomics of, in Burma, **243–267.**
Anopheles amictus hilli, habits and behaviour of, in Queensland, **159–177.**
Anopheles annularis, bionomics of, in Burma, **243–267.**
Anopheles annulipes, and malaria in Australasia, **176.**
Anopheles bancrofti, breeding places of, in Queensland, **159, 176.**
Anopheles barbirostris, bionomics of, in Burma, **243–267.**
Anopheles coustani var. *ziemanni*, breeding places of, in Sudan, **143–152.**
Anopheles culicifacies, bionomics of, in Burma, **243–267**; in India, **175.**
Anopheles culicifacies var. *adenensis*, characters and bionomics of, in Socotra, **225.**
Anopheles dthali var. *wardi*, n., from Socotra, **223.**
Anopheles fluviatilis, in India, **175**; systematic notes on, **263–264.**
Anopheles fuliginosus (see *A. annularis*).
Anopheles funestus, bionomics of, in E. Africa, **453–465**; breeding places of, in Sudan, **143–152**; fungus infesting, in Uganda, **208.**
Anopheles gambiae, in Africa, **175**; bionomics of, in East Africa, **453–465**; breeding places and control of, in Sudan, **143–153**; fungus infesting, in Uganda, **208.**
Anopheles hyrcanus (& var. *nigerrimus*), bionomics of, in Burma, **243–267.**
Anopheles jeyporiensis (& var. *candidiensis*), bionomics of, in Burma, **243–267.**
Anopheles kochi, bionomics of, in Burma, **243–267.**
Anopheles leucosphyrus, bionomics of, in Burma, **243–267**; status of, as vector of malaria, **254–256.**
Anopheles maculatus, bionomics of, in Burma, **243–251.**
Anopheles maculipennis var. *indiensis* (see *A. splendidus*).
Anopheles minimus, in India, **175**; bionomics of, in Burma, **243–262**; status of, as vector of malaria, **256**; systematic notes on, **262–265.**
Anopheles nili, breeding places of, in Sudan, **143–152.**
Anopheles pallidus, systematic notes on, **262.**
Anopheles pharoensis, breeding places and control of, in Sudan, **143–153**; bionomics and possible relation of, to malaria, **150–152.**
Anopheles philippinensis, bionomics of, in Burma, **243–262**; systematic notes on, **262.**
Anopheles punctulatus, habits and behaviour of, in New Guinea, **169–177.**
Anopheles punctulatus farauti, habits and behaviour of, in Queensland and New Guinea, **159–177.**
Anopheles rossii (see *A. subpictus*).
Anopheles rufipes, breeding places, bionomics and control of, in Sudan, **143–153.**
Anopheles splendidus, in Burma, **243.**
Anopheles squamosus, breeding places of, in Sudan, **143–152.**
Anopheles stephensi, bionomics of, in Burma, **243–249.**
Anopheles subpictus, bionomics of, in Burma, **243–267**; in New Guinea, **172, 173.**
Anopheles sundaicus, in Burma, **259.**
Anopheles tessellatus, bionomics of, in Burma, **243–267.**
Anopheles vagus, bionomics of, in Burma, **243–267.**
Anopheles varuna, systematic notes on, **264–265.**
Anopheles wellcomei, breeding places of, in Sudan, **143–152.**
antennatus, *Culex*; *Tribolium*.
Anthophora, *Tribolium confusum* in nests of, **16.**
Apanteles bordagei, parasite of *Leucoptera* in E. Africa, **406–412**; bionomics of, **412.**
Aphanotus (see *Tribolium*).
Aphis laburni (*leguminosae*, auct.), transmitting rosette disease of groundnuts in Tanganyika, **233.**
apicicornis, *Coryna*.
Aplocheilichthys loati, predacious on mosquitos in Sudan, **141.**
appendiculatus, *Rhipicephalus*.
Apple, Coccinellids predacious on mites on, **299.**
aptus, *Stethorus*.

Arabia, Coccinellid in, **311** ; *Glossina tachinoides* in, **529.**
arabiensis, Aëdes (*Aëdimorphus*).
ares, Tribolium indicum.
argenteus, Aëdes.
Argentina, new *Tribolium* from, **32.**
argentipes, Phlebotomus (*Euphlebotomus*).
armigera, Heliothis.
Arsenic, against ticks, **269.**
Arthroleptis, and mosquitos in Uganda, **211.**
articollis, Systates.
Ascogaster quadridentatus, parasite of *Laspeyresia nigricana* in Britain, **67.**
Ascotis reciprocaria, on groundnuts in Uganda, **233.**
atomus, Stethorus.
Atoposoma variegatum var. *afra*, parasite of *Leucoptera* in E. Africa, **406–412** ; bionomics of, **412.**
Australia, habits and behaviour of *Anopheles* in, **159–177** ; Coccinellids in, **316** ; new *Tribolium* from, **51, 55.**
autumnalis, Trombicula.
Avocado, Coccinellid predacious on mite on, in U.S.A., **299.**

B.

balfouri, Uranotaenia.
bancrofti, Anopheles.
Banksinella (see *Aëdes*).
barbirostris, Anopheles.
Barbus stygmatopygus, predacious on mosquitos in Sudan, **141.**
Beans, Madagascar, new Jassid causing leaf-crinkle of, in Fiji, **131.**
Beet, Coccinellid predacious on mite on, in Italy, **299.**
Bemisia tabaci, on groundnuts in Belgian Congo, **233.**
benningseni, Ootheca.
Bent (see *Molinia caerulea*).
Benzene Hexachloride (Gammexane), experiments with, against insects and ticks, **83, 179–183, 269–276, 277–279, 423–434.**
bicolor, Ergana.
bifasciata, Acrocercops.
bifidus, Stethorus.
bimaculatus, Tetranychus (see *T. telarius*).
Biological Control, determination of value of, **435–440.**
bitaeniorhynchus, Culex.
Blatta orientalis, effect of temperature on, **353.**
Bohemia, Coccinellid predacious on mite on hops in, **299.**
Boophilus (*Palpoboophilus*) *decoloratus*, DDT and "Gammexane" against, on cattle in E. Africa, **269, 424–427.**
Boophilus fallax, on cattle, **269.**
borboricus, Eulophus.
bordagei, Apanteles.
bovis, Hypoderma.
Brachylaena hutchinsonii, **489.**
Bracken (*Pteris aquilinia*), relation of, to tick ecology in Wales, **331–337.**
brevicornis, Tribolium.
brevifilis, Phlebotomus (*Anaphlebotomus*).
brevipes, Pseudococcus.
brevis, Stethorus.
breviuscula, Rodolia.
Britain, Coccinellid predacious on mites in, **299**; ecology of *Ixodes ricinus* in, **321–337** ; bionomics and control of *Laspeyresia nigricana* in, **57–87** ; methods of trapping and rearing mites in, **281–295.**
brumpti, Brumptomyia.
Brumptomyia, status of, **94.**
Brumptomyia brumpti, type of genus, **104.**
Bryobia praetiosa, Coccinellid predacious on, in Tasmania, **299.**
Bucolus, **302.**
Burma, mosquitos and malaria in, **237–268.**

C.

Cacao, development of *Ephestia elutella* on, **121–124.**
caffeina, Leucoptera.
Calodendron capense, **489.**
canadensis, Polistes.
candidiensis, Anopheles jeyporiensis.
canisuga, Ixodes.
Canna orientalis, mosquitos breeding in, in Uganda, **192–211.**
Cape Chestnut (see *Calodendron capense*).
cardinalis, Rodolia.
carinatum, Tribolium.
Cassia, pineapples in plantations of, **191.**
castaneum, Tribolium.
Castor, Coccinellids predacious on mites on, **299.**
Catamerus revoili, on groundnuts in Tanganyika, **234.**
Cattle, *Ixodes ricinus* on, in Wales, **321** ; experiments with DDT and "Gammexane" against, in Uganda, **269–276, 277–279, 423–434.**
caudatus, Ephialtes.
cautella, Ephestia.
ceratopogones, Chaoborus.

ceto, *Tribolium*.
Ceylon, *Rodolia breviuscula* in, **536**.
Chaetonodexodes marshalli, parasite of *Schematiza cordiae* in Trinidad, **218, 439**.
Chaoborus ceratopogones, in Sudan, **143, 149**.
Chara, Anopheline larvae associated with, **224**.
China, new Coccinellid in, **314**.
chinensis, *Phlebotomus* (*Adlerius*).
Chrotogonus rotundatus, on groundnuts in Tanganyika, **232**.
Chrysocharis lepelleyi, parasite of *Leucoptera* in E. Africa, **406, 410**; bionomics of, **413**.
chrysogaster, *Eretmapodites*.
chrysosoma, *Taeniorhynchus* (*Coquillettidia*).
cinctiventris, *Cirrospilus*.
circumluteolus, *Aëdes* (*Banksinella*).
Cirrospilus cinctiventris, parasite of *Leucoptera* in E. Africa, **406**; bionomics of, **414**.
citriculus, *Pseudococcus*.
Citrus, Coccinellid predacious on mites on, in U.S.A., **299**.
Clethrionomys glareolus, tick on, in Wales, **321**.
Closterocerus africanus, parasite of *Leucoptera* in E. Africa, **410**; bionomics of, **413**.
Coelomyces, infesting *Anopheles* in Uganda, **208**.
coffeae, *Derostenus*.
Coffee, bionomics of *Leucoptera coffeella* on, in Tanganyika, **399–416**.
Coffee Leaf Miner (see *Leucoptera coffeella*).
coffeella, *Leucoptera*.
coffeicola, *Pleurotropis*.
Colobus, **489**.
Colocasia esculentum (*antiquorum*), mosquitos breeding in, in Uganda, **190–195**.
Colombia, new Coccids on bananas from, **419–420**.
colombianus, *Pseudococcus*.
comstocki, *Pseudococcus*.
confusum, *Tribolium*.
congesta, *Zophosis*.
Congo, Belgian, new *Tribolium* from, **35**.
Coquillettidia (see *Taeniorhynchus*).
Cordia macrostachya, effect of defoliation on reproduction of, **213–215**; biological control of, in Mauritius, **213, 217, 436**.
cordiae, *Schematiza*.
Coreopsis, *Hilda patruelis* on, in E. Africa, **233**.
cornutus, *Gnathocerus*.
Coryna spp., on groundnuts in Tanganyika, **234**.
Cotton, Coccinellids predacious on mites on, **299**; relation between hairiness and Jassid resistance of, **539–575**.
coustani, *Anopheles*.
Cowpeas, new Jassid causing leaf-crinkle of, in Fiji, **131**.
Craibia elliottii, mosquitos associated with, in Kenya, **490**.
Cricetus auratus, mites on, **284**.
Croton megalocarpon, **489**.
cruralis, *Stethorus*.
Cryptolaemus, **302**.
Cryptus inornatus, parasite of *Loxostege* in N. America, **438**.
Cryptus sexannulatus, parasite of *Cydia pomonella* in S. France, **438**.
Culex, in E. Africa, **454**; key to adults of, in Socotra, **228**.
Culex antennatus, in Sudan, **143, 146**.
Culex bitaeniorhynchus, breeding places of, in Sudan, **143–146**.
Culex decens, in Sudan, **143–146**.
Culex ethiopicus, breeding places of, in Sudan, **143–146**.
Culex fatigans, in Socotra, **228**; effect of humidity on, **352, 353**.
Culex laticinctus, in Socotra, **228**.
Culex musarum, breeding in plant axils in Uganda, **200–210**.
Culex nebulosus, in Sudan, **143, 152**; breeding in plant axils in Uganda, **200–210**.
Culex perfuscus, in Sudan, **143–146**.
Culex pipiens (& subsp. *molestus*), in Sudan, **143**.
Culex poicilipes, breeding places of, in Sudan, **143–154**.
Culex simpliforceps, in Sudan, **143**.
Culex sinaiticus, in Socotra, **228**.
Culex sitiens, larva of, in Socotra, **227**.
Culex thalassius, in Socotra, **228**.
Culex tigripes, in Sudan, **143, 152**.
Culex toroensis, breeding in plant axils in Uganda, **200–210**.
Culex trifilatus, breeding in plant axils in Uganda, **200–210**.
Culex univittatus (& var. *neavei*), breeding places of, in Sudan, **143–146**.
culicifacies, *Anopheles* (*Myzomyia*).
Culiciomyia (see *Culex*).
Cydia pomonella, parasites of, in S. France, **438**.
Cydnus, on groundnuts in Tanganyika, **232**.
cylindricum, *Tribolium*.
Cynodon dactylon, army worms on, in E. Africa, **234**.
Cyprus, Coccinellid predacious on red spider in, **304**.

D.

daricella, Leucoptera.
darwini, Stethorus (Nephopullus).
DDT, experiments with, against insects and ticks, **72–80, 179–183, 269–276, 277–279, 423–434.**
deboeri, Aëdes (Stegomyia).
decens, Culex.
decoloratus, Boophilus (Palpoboophilus).
deliensis, Trombicula.
dentatus, Aëdes.
dentipes, Alcidodes (Alcides).
Dereodus recticollis, on groundnuts in Nyasaland, **235.**
Dermacentor reticulatus, hosts of, in Wales, **321.**
Derostenus coffeae, parasite of *Leucoptera* in E. Africa, **410** ; bionomics of, **413.**
destructor, Tribolium.
devastans, Empoasca.
Diaecoderus, on groundnuts in Tanganyika, **235.**
Diceromyia (see *Aëdes*).
differentialis, Melanoplus.
dinota, Tortrix.
dolichi, Empoasca.
dolon, Tribolium.
domestica, Musca.
downesi, Tribolium.
dracaenae, Eretmapodites.
Drosichiella, Rodolia breviuscula predacious on, in India, **536.**
drosophilae, Spalangia.
dthali, Anopheles (Myzomyia).
dubium, Tribolium carinatum.

E.

East Coast Fever, ticks associated with, on cattle, **269, 424–433.**
Elasmus leucopterae, parasite of *Leucoptera* in E. Africa, **406, 410** ; bionomics of, **412.**
elisae, Pseudococcus.
elutella, Ephestia.
Emmallus major, on groundnuts in Tanganyika, **234.**
Empoasca devastans, on cotton in India, **540, 569.**
Empoasca dolichi, on groundnuts in Uganda, **232.**
Empoasca facialis, hairiness of cotton and resistance to, **539–575.**
Ephestia cautella, in stored groundnuts in E. Africa, **235.**
Ephestia elutella, development of, on natural foods, **117–130.**
Ephestia kühniella, **128.**
Ephialtes caudatus, parasite of *Cydia pomonella* in S. France, **438.**
Epiplatys marnoi, predacious on mosquitos in Sudan, **142.**
Eretmapodites spp., breeding in plant axils in Uganda, **196–210.**
Ergana bicolor, on groundnuts in Tanganyika, **234.**
Erica tetralix (see Heather).
Eriophorum polystachyon (Cotton Grass), relation of, to tick ecology in Wales, **331–337.**
Erythroneura leveri, sp. n., vector of leaf-crinkle of beans and cowpeas in Fiji, **131.**
Estigmene unipunctata, on groundnuts in Uganda, **233.**
ethiopicus, Culex.
Etorleptiomyia (see *Ficalbia*).
Eulophus spp., bionomics of, as parasites of *Leucoptera* in E. Africa, **406, 408, 413, 414.**
Euphlebotomus, a new subgenus of *Phlebotomus,* **98.**
Eupropacris, possibly associated with groundnuts in Tanganyika, **232.**
Eutrombicula alfreddugesi, collection of larvae of, **281.**
evertsi, Rhipicephalus.
Evotomys glareolus (see *Clethrionomys*).
exempta, Laphygma.
exigua, Laphygma.
exsiccator, Tetranychus.

F.

facialis, Empoasca.
fallax, Boophilus.
farauti, Anopheles punctulatus.
fatigans, Culex.
ferox, Eretmapodites.
Ficalbia spp., in Sudan, **143, 146.**
Figs, development of *Ephestia elutella* on, **120–124.**
Fiji, new Coccinellid from, **319** ; new Jassid causing leaf-crinkle of beans and cowpeas in, **131.**
fijiensis, Stethorus.
Finland, Coccinellid predacious on mite on fruit trees in, **299.**
Finlaya (see *Aëdes*).
Fish, species of, predacious on mosquitos in Sudan, **141–142, 153.**
Flour, Soya, development of *Ephestia elutella* on, **120–124.**
fluviatilis, Anopheles.
freemani, Tribolium.
frit, Oscinella.
fuliginosus, Anopheles (see *A. annularis*).

fumida, Rodolia.
funestus, Anopheles (Myzomyia).
fuscipes, Glossina palpalis.

G.

gambiae, Anopheles (Myzomyia).
Gambusia affinis holbrooki, and mosquitos in Sudan, **153.**
Gammexane (see Benzene Hexachloride).
gebieni, Tribolium.
gestroi, Peristeptus.
giganteum, Tribolium.
gigas, Phlebotomus (Spelaeophlebotomus).
gilvifrons, Stethorus.
globulus, Sphrigodes.
Glossina longipalpis, traps for, in W. Africa, **492.**
Glossina morsitans, in Sudan, **529**; traps for, in W. Africa, **491–528**; isolated generations of, **441–451.**
Glossina pallidipes, traps for, in W. Africa, **491–528**; isolated generations of, **441–451.**
Glossina palpalis, traps for, in W. Africa, **491–528**; fed on cattle treated with DDT and "Gammexane", **423–433.**
Glossina palpalis subsp. *fuscipes*, in Sudan, **529.**
Glossina swynnertoni, isolated generations of, **441–451.**
Glossina tachinoides, traps for, in W. Africa, **491–528**; in Sudan, **529**; distribution of, **529.**
Glypta haesitator, parasite of *Laspeyresia nigricana* in Britain, **67.**
Gnathocerus spp., **24.**
Gossypium (see Cotton).
gracillima, Rhytinota.
granti, Aëdes (Stegomyia).
Grasses, Coccinellid predacious on mite on, in Tasmania, **299.** (See also *Agrostis* and *Molinia*.)
Grass, Cotton (see *Eriophorum polystachyon*).
gregaria, Schistocerca.
Groundnuts, annotated list of insects associated with, in E. Africa, **231–236**; development of *Ephestia elutella* on, **121–124.**
guerini, Rodolia.
Guineapigs, as bait for mites, **285.**

H.

Haemaphysalis, in Wales, **321.**
haesitator, Glypta.
Hallirhotius africana, in Uganda, **235.**
Haplochromis multicolor, predacious on mosquitos in Sudan, **142.**
Harpagomyia taeniarostris, breeding in plant axils in Uganda, **195–210.**
Hawaii, Coccinellid predacious on mite on sugar-cane in, **299.**
haworthi, Aëdes.
Heather (*Erica tetralix*), relation of, to tick ecology in Wales, **331–337.**
Helicopter, equipment for applying sprays from, **1–12.**
Heliothis armigera, on groundnuts in N. Rhodesia, **234**; a potential pest in Tanganyika, **234.**
Hemisus marmoratus, and mosquitos in Uganda, **211.**
Hemiteles, parasite of *Leucoptera* in E. Africa, **410.**
Hemiteles ridibundus, parasite of *Laspeyresia nigricana* in Britain, **67.**
Hemp, Bowstring (see *Sansevieria*).
hexagonus, Ixodes.
Hilda patruelis, bionomics of, on groundnuts in E. Africa, **233.**
hilli, Anopheles amictus.
hispida, Ficalbia (Mimomyia).
Hops, Coccinellid predacious on mites and Aphid on, **299.**
Horaiella, status of, **95.**
Horismenus, parasite of *Physonota*, **436.**
Hormius, species of genus allied to, parasite of *Leucoptera* in E. Africa, **406, 408**; bionomics of, **414.**
Horses, *Ixodes ricinus* on, in Wales, **321.**
hospitii, Sergentomyia (Sintonius).
Humidity, influence of, on *Musca domestica*, **339–357.**
humilis, Iridomyrmex.
humuli, Phorodon.
Hyalomma impressum, on cattle, **269.**
Hyperolius, and mosquitos in Uganda, **211.**
Hypoderma spp., **335.**
hyrcanus, Anopheles.

I.

Icerya aegyptiaca, Rodolia breviuscula predacious on, in India, **536.**
Icerya purchasi, new Coccinellids predacious on, in India and Ceylon, **536–538.**
impressum, Hyalomma.
India, Coccinellids predacious on mites in, **304, 310, 312, 313**; *Empoasca devastans* on cotton in, **540, 569**; revision of *Rodolia* species of, **531–538**; new *Tribolium* from, **50.**

indicum, *Tribolium*.
indicus, *Paratetranychus*.
indiensis, *Anopheles maculipennis* (see *A. splendidus*).
ingrami, *Aëdes* (*Finlaya*).
Ingramia (see *Ficalbia*).
inornatus, *Cryptus* ; *Eretmapodites*.
investigatorum, *Spilosa*.
Iridomyrmex humilis, *Tribolium myrmecophilum* in nests of, **21.**
Italy, Coccinellid predacious on mites in, **299.**
Ixodes canisuga, in Wales, **321.**
Ixodes hexagonus, in Wales, **321.**
Ixodes putus (see *I. uriae*).
Ixodes ricinus, on sheep, **269** ; ecology of, in Glamorgan, **321–337.**
Ixodes trianguliceps (*tenuirostris*), hosts of, in Wales, **321.**
Ixodes unicavatus, in Wales, **321.**
Ixodes uriae, in Wales, **321.**
Ixodes vespertilionis, in Wales, **321.**

J.

Jassids, relation between hairiness of cotton and resistance to, **539–575.**
jejunus, *Stethorus*.
jeyporiensis, *Anopheles*.
Juncus communis (see Rush).
Jute, Coccinellid predacious on mite on, in India, **299.**

K.

Kassina senegalensis, and mosquitos in Uganda, **211.**
keniensis, *Aëdes* (*Stegomyia*).
Kenya Colony, habit of mosquitos in forests in, **489–490** ; new *Tribolium* from, **39.**
kochi, *Aëdes* ; *Anopheles*.
kühniella, *Ephestia*.

L.

laburni, *Aphis*.
lacustris, *Ficalbia* (*Mimomyia*).
lanuginosa, *Coryna*.
Laphygma spp., on groundnuts in E. Africa, **234.**
Larat Island, new *Tribolium* from, **52.**
Larroussius, a subgenus of *Phlebotomus*, **97.**
Laspeyresia nigricana, bionomics and control of, in Britain, **57–84.**
Lathyrus spp. (Sweet & Meadow Peas), *Laspeyresia nigricana* on, in Britain, **67.**
laticinctus, *Culex*.
Leanum (see *Tribolium*).
leguminosae, auct., *Aphis* (see *A. laburni*).
Lemon, Coccinellid predacious on mite on, in Sicily, **299.**
lepelleyi, *Chrysocharis*.
Leptopelis johnstoni, and mosquitos in Uganda, **211.**
Leucoptera caffeina, characters and bionomics of, on coffee in Tanganyika, **399, 404.**
Leucoptera coffeella, bionomics and parasites of, in Tanganyika, **399–416.**
Leucoptera daricella, status of, **399.**
leucoptera, *Mirax*.
leucopterae, *Elasmus* ; *Tetrastichodes*.
leucopus, *Eretmapodites*.
leucosphyrus, *Anopheles*.
leveri, *Erythroneura*.
limbirena, *Plusia*.
lineatum, *Hypoderma*.
linsleyi, *Tribolium*.
Liogryllus morio, on groundnuts in Tanganyika, **231.**
litura, *Prodenia*.
Locusta migratoria migratorioides, on groundnuts in Tanganyika, **231.**
longipalpis, *Glossina* ; *Lutzomyia*.
Loxostege sticticalis, parasite of, in N. America, **438.**
Lucilia, effect of temperature on activity of, **350.**
Luperodes quaternus, on groundnuts in Uganda, **235.**
Lutzia (see *Culex*).
Lutzomyia, status of, **94.**
Lutzomyia longipalpis, type of genus, **104.**
Lyphia, distribution and characters of, **15, 23–25.**

M.

Macropoda nigrogemmata, on groundnuts in Tanganyika, **234.**
maculatus, *Anopheles*.
maculipennis, *Anopheles*.
Madagascar, Coccinellid in, **308, 534** ; new *Tribolium* spp. from, **47–49.**
Madeira, new Coccinellid from, **305.**
madens, *Tribolium*.
major, *Emallus* ; *Phlebotomus* (*Larroussius*).

Malaria, and mosquitos: in Burma, **237–268**; in Socotra, **225**; in Sudan, **150–152**.
Malaya, new Coccinellids from, **315, 316**; new *Tribolium* from, **53**.
Man, reactions of *Aëdes aegypti* to stimuli associated with, **387–397**.
Mansonioides (see *Taeniorhynchus*).
maritimus, *Pseudococcus*.
Markhamia, pineapple in plantations of, **191**.
marshalli, *Aëdes* (*Aëdimorphus*); *Chaetonodexodes*.
martini, *Phlebotomus* (*Synphlebotomus*).
Maruca testulalis, on groundnuts in Uganda, **233**.
mashonaensis, *Uranotaenia*.
Mashonaland, new *Tribolium* from, **35**.
mauritanicus, *Tenebroides*.
Mauritius, new Coccinellid from, **309**; biological control of *Cordia* in, **213, 217, 436**.
maxillosus, *Gnathocerus*.
Meadow Pea (see *Lathyrus pratensis*).
mediolineata, *Ficalbia* (*Etorleptiomyia*).
Megalixalus fulvovittatus, and mosquitos in Uganda, **211**.
Melanagromyza, parasites of, in E. Africa, **413, 414**.
Melanoplus differentialis, **485**.
Melea, pineapple in plantations of, **191**.
metallicus, *Aëdes* (*Stegomyia*); *Taeniorhynchus* (*Coquillettidia*).
Mexico, new *Tribolium* from, **34**.
Micralestes acutidens, predacious on mosquitos in Sudan, **141**.
Microtus orcadensis, mites on, **284**.
migratoria, *Locusta*.
migratorioides, *Locusta migratoria*.
Mimomyia (see *Ficalbia*).
mimomyiaformis, *Ficalbia* (*Mimomyia*).
minima, *Rodolia*.
minimus, *Anopheles*.
minuta, *Sergentomyia*.
minutissimus, *Stethorus*.
mirabilis, *Sergentomyia* (*Spelaeomyia*).
Mirax leucoptera, hosts of, in E. Africa, **399**.
Mites, revision of Old World species of *Stethorus* predacious on, **297–319**; methods of trapping and rearing, **281–295**.
Mochthogenes (see *Culex*).
molestus, *Culex pipiens*.
Molinia caerulea, relation of, to tick ecology in Wales, **331–337**.
moloneyi, *Anaphe*.
morio, *Liogryllus*.
morogorensis, *Allodontermes*.
morsitans, *Glossina*.
Mosquitos, bionomics of, in E. Africa, **453–465**; and malaria in Burma, **237–268**; habits of, in forests in Kenya, **489–490**; of Socotra I., **221–229**; of Jebel Auliya Reservoir, Sudan, **133–157**; toxicity of limewash containing DDT and "Gammexane" to, **179–183**; reaction of, to stimuli, **387–397**.
moubata, *Ornithodoros*.
Musa spp., mosquitos breeding in, in Uganda, **187–189**.
musarum, *Culex*; *Uranotaenia ornata*.
Musca domestica, influence of climatic factors on, **339–357**; method of breeding, in laboratory, **385–386**.
myrmecophilum, *Tribolium* (*Leanum*).
Myzomyia (see *Anopheles*).

N.

neavei, *Culex univittatus*.
nebulosus, *Culex* (*Culiciomyia*).
Neophlebotomus, a synonym of *Sergentomyia*, **89**.
Nephopullus (see *Stethorus*).
nepoides, *Sphaerodema*.
netara, *Rodolia*.
New Guinea, habits and behaviour of *Anopheles* in, **159–177**.
New Zealand, new Coccinellid predacious on mites in, **318**.
Nigeria, new *Tribolium* from, **35**.
nigerrimus, *Anopheles hyrcanus*.
nigricana, *Laspeyresia*.
nigripes, *Stethorus*.
nigrogemmata, *Macropoda*.
nili, *Anopheles* (*Myzomyia*).
Nomadacris septemfasciata, on groundnuts in E. Africa, **232**.

O.

Oats, development of *Ephestia elutella* on, **120–124**.
ochraceus, *Aëdes* (*Aëdimorphus*).
ogloblini, *Stethorus*.
Olea chrysophylla (Olive), **489**.
Ootheca benningseni, in Uganda, **235**.
orientalis, *Blatta*.
ornata, *Pogonobasis*; *Uranotaenia*.
Ornithodoros moubata, **479**.
Oscinella frit, parasite of, in N. America, **436**.
Osmia, *Tribolium confusum* in nests of, **16**.

P.

P530 (see Gammexane).
pacificus, Tetranychus.
pallescens, Piezodorus.
pallidipes, Glossina.
pallidus, Anopheles.
palpalis, Glossina.
Palpoboophilus (see *Boophilus*).
Pandanus chiliocarpus, mosquitos breeding in, in Uganda, **191–211.**
papatasi, Phlebotomus.
parallelus, Tribolium.
Paraphlebotomus, new subgenus of *Phlebotomus*, **97.**
Parasa vivida, on groundnuts in Tanganyika, **234.**
Paratetranychus indicus, Coccinellid predacious on, on sorghum in India, **299.**
Paratetranychus pilosus, **486** ; food-plants and predators of, **299.**
parcepunctatus, Stethorus.
parki, Tribolium.
parva, Theileria.
patruelis, Hilda.
pauperculus, Stethorus.
Pavetta ternifolia, Leucoptera on, in E. Africa, **399.**
Pea Moth (see *Laspeyresia nigricana*).
Peas, development of *Ephestia elutella* on, **121–124** ; bionomics and control of *Laspeyresia nigricana* on, in Britain, **57–87.**
peregrinabundus, Pseudococcus.
perfuscus, Culex.
Pericoma townsvillensis, **95.**
Peristeptus gestroi, on groundnuts in Tanganyika, **234.**
pharoensis, Anopheles (*Myzomyia*).
Phaseolus lunatus (see Beans).
philippinensis, Anopheles.
Phlebotominae, classification of Old World species of, **85–115.**
Phlebotomus, characters of, **92** ; scope of subgenera of, **96–100.**
Phlebotomus argentipes, type of new subgenus *Euphlebotomus*, **98.**
Phlebotomus brevifilis, type of new subgenus *Anaphlebotomus*, **99.**
Phlebotomus chinensis, type of subgenus *Adlerius*, **98.**
Phlebotomus major, type of subgenus *Larroussius*, **97.**
Phlebotomus martini, type of new subgenus *Synphlebotomus*, **97.**
Phlebotomus papatasi, type of genus, **96.**
Phlebotomus sergenti, type of new subgenus *Paraphlebotomus*, **97.**
Phorodon humuli, Coccinellid predacious on, on hops in Britain, **299.**
Physonota alutacea, proposed introduction of, into Mauritius against *Cordia*, **213** ; parasite of, **436.**
picipes, Stethorus.
Piezodorus pallescens, on groundnuts in Uganda, **232.**
pilosus, Paratetranychus.
Pineapple (*Ananas comosus*), mosquitos breeding in, in Uganda, **191–195.**
pipiens, Culex.
Platybolium, **25, 26.**
Pleurotropis spp., parasites of *Leucoptera* in E. Africa, **406, 408, 410** ; bionomics of, **413.**
plumosa, Ficalbia (*Mimomyia*).
Plusia spp., on groundnuts in E. Africa, **234.**
Pogonobasis ornata, on groundnuts in Tanganyika, **234.**
poicilipes, Culex.
Polistes canadensis, predacious on *Schematiza cordiae* in Trinidad, **217.**
politum, Tribolium.
pomonella, Cydia.
Populations, methods of sampling field, **435–440.**
praetiosa, Bryobia.
Prodenia litura, on groundnuts in Uganda, **234.**
productus, Eretmapodites leucopus.
prolixus, Rhodnius.
Promecotheca reichei, **414.**
Prophlebotomus, a synonym of *Sergentomyia*, **88.**
Pseudococcus brevipes, on groundnuts in E. Africa, **233.**
Pseudococcus citriculus, characters of, **418.**
Pseudococcus colombianus, sp. n., on bananas from Colombia, **420.**
Pseudococcus comstocki, key to allied spp. and females of, **417** ; characters of, **418.**
Pseudococcus elisae, sp. n., on bananas from Colombia, **419.**
Pseudococcus maritimus, characters of, **417.**
Pseudococcus peregrinabundus, sp. n., on bananas from Colombia, **419.**
Pteris aquilina (see Bracken).
pulchrithorax, Aëdes (*Finlaya*).
punctillum, Stethorus.
punctulatus, Anopheles.
punctum, Stethorus.
purchasi, Icerya.
putus, Ixodes (see *I. uriae*).

Q.

quadridentatus, Ascogaster.
quarternus, Luperodes.
quinquevittatus, Eretmapodites.

R.

rani, *Stethorus*.
reciprocaria, *Ascotis*.
recticollis, *Dereodus*.
reichei, *Promecotheca*.
reticulatus, *Dermacentor*.
revoili, *Catamerus*.
Rhipicephalus appendiculatus, experiments with DDT and "Gammexane" against, **269–276, 277–279, 423–431.**
Rhipicephalus evertsi, on cattle in Uganda, **269, 424–427.**
Rhipicephalus simus, on cattle in Uganda, **269.**
Rhodnius prolixus, permeability of chorion and sub-chorial membranes of, **359–383, 467–488**; structure of egg-shell of, **359.**
Rhytinota spp., on groundnuts in Tanganyika, **234.**
Rice, development of *Ephestia elutella* on, **120–124.**
ricinus, *Ixodes*.
ridibundus, *Hemiteles*.
Riouw Archipelago, new *Tribolium* from, **54.**
ritchiei, *Achrysocharella*.
Rodolia, revision of Indian species of, **531–538.**
Rodolia amabilis, sp. n., predacious on *Icerya purchasi* in India, **536.**
Rodolia andamanica, characters of, in Andaman Is., **535.**
Rodolia breviuscula, characters of, in India and Ceylon, **536.**
Rodolia cardinalis, predacious on *Icerya purchasi*, **531**; characters of, **532.**
Rodolia fumida, characters, distribution and hosts of, **534.**
Rodolia guerini, characters and synonymy of, in India, **535.**
Rodolia minima, sp. n., on *Icerya purchasi* in India, **537.**
Rodolia netara, sp. n., on *Icerya purchasi* in India, **537.**
Rodolia ruficollis, characters of, in India, **535.**
Rodolia rufopilosa, probably not an Indian species, **533.**
Rodolia sexmaculata (see *R. guerini*).
Rosette Disease of Groundnuts, and *Aphis laburni*, **233.**
rossii, *Anopheles* (see *A. subpictus*).
rotundatus, *Chrotogonus*; *Stethorus*.
rotundulus, *Stethorus*.
ruficollis, *Rodolia*.
rufipes, *Anopheles* (*Myzomyia*).
rufopilosa, *Rodolia*.
Rush, relation of, to tick ecology in Wales, **331–337.**
russeolus, *Tetranychus telarius*.

S.

salutaris, *Stethorus*.
Sambucus nigra, mite on, in Europe, **299.**
samoana, *Aëdes kochi*.
Sansevieria spp. (Bowstring Hemp), mosquitos breeding in, in Uganda, **191.**
Sardinia, Coccinellid predacious on red spider in, **304.**
Schematiza cordiae, proposed introduction of, into Mauritius against *Cordia*, **213**; control of, by parasites in Trinidad, **217–220, 439.**
Schistocerca gregaria, on groundnuts in E. Africa, **232.**
semele, *Tribolium*.
Senegal, new *Tribolium* from, **35, 44.**
septemfasciata, *Nomadacris*.
seres, *Tribolium indicum*.
sergenti, *Phlebotomus* (*Paraphlebotomus*).
Sergentomyia, status of, **92**; synonymy of, **88**; characters of, **93**; groups and sub-genera of, **101–103.**
Sergentomyia hospitii, type of subgenus *Sintonius*, **103.**
Sergentomyia minuta, type of genus, **100.**
Sergentomyia mirabilis, type of new subgenus *Spelaeomyia*, **103.**
sexannulatus, *Cryptus*.
sexmaculata, *Rodolia* (see *R. guerini*).
Sheep, *Ixodes ricinus* on, in Wales, **321.**
Sierra Leone, new Coccinellid predacious on mites from, **306.**
simpliforceps, *Culex* (*Mochthogenes*).
simpsoni, *Aëdes* (*Stegomyia*).
simus, *Rhipicephalus*.
sinaiticus, *Culex*.
Sintonius, a subgenus of *Sergentomyia*, **103.**
siphonulus, *Stethorus*.
sitiens, *Culex*.
Sleeping Sickness, trapping *Glossina* for control of, in W. Africa, **516–525.**
Socotra Island, mosquitos of, **221–229.**
Soya Flour (see Flour).
Spalangia drosophilae, parasite of *Oscinella frit* in N. America, **436.**
Spelaeomyia, a new subgenus of *Sergentomyia*, **103.**
Spelaeophlebotomus, a new subgenus of *Phlebotomus*, **100.**
Sphaerodema nepoides, predacious on mosquitos in Sudan, **141.**
Sphrigodes globulus, on groundnuts in Tanganyika, **235.**
Spilochalcis, parasite of *Chaetonodexodes marshalli* in Trinidad, **218.**

Spilosoma investigatorum, on groundnuts in Uganda, **233.**
Spirogyra, Anopheline larvae associated with, **224.**
splendens, *Ficalbia* (*Mimomyia*).
splendidus, *Anopheles*.
Sprays, development of helicopter for, **1–12.**
squamosus, *Anopheles* (*Myzomyia*).
Stegomyia (see *Aëdes*).
stephensi, *Anopheles*.
Stethorus, revision of Old World species of, **297–320.**
Stethorus aethiops, characters and distribution of, **306.**
Stethorus aptus, sp. n., from China, **314.**
Stethorus bifidus, sp. n., predacious on mites in New Zealand, **317.**
Stethorus cruralis, **309.**
Stethorus fijiensis, sp. n., from Fiji, **318.**
Stethorus gilvifrons, hosts and distribution of, **299**; characters of, **303.**
Stethorus jejunus, characters and distribution of, **307.**
Stethorus minutissimus, characters of, in Madagascar, **308.**
Stethorus nigripes, sp. n., from W. Australia, **316.**
Stethorus parcepunctatus, sp. n., from India, **312.**
Stethorus pauperculus, characters, distribution and hosts of, **299, 309, 310.**
Stethorus punctillum, characters, hosts and distribution of, **299, 302.**
Stethorus rani, sp. n., from India, **313.**
Stethorus rotundatus, status of, **311.**
Stethorus rotundulus, status of, **311.**
Stethorus salutaris, sp. n., predacious on mites in Sierra Leone, **306.**
Stethorus siphonulus, sp. n., from Malaya, **314.**
Stethorus tetranychi, sp. n., predacious on red spider in India, **311.**
Stethorus truncatus, sp. n., from Malaya, **315.**
Stethorus vagans, hosts, distribution and characters of, **299, 316.**
Stethorus vinsoni, sp. n., from Mauritius, **309.**
Stethorus wollastoni, sp. n., from Madeira, **305.**
sticticalis, *Loxostege*.
Stimuli, reactions of *Aëdes aegypti* to, **387–397.**
subpictus, *Anopheles*.
Sudan, Anglo-Egyptian, new *Tribolium* from, **44**; mosquitos of Jebel Auliya Reservoir in, **133–157.**
Sudan, French (see Africa, French West).
Sugar-cane, Coccinellid predacious on mite on, in Hawaii, **299.**
sulmo, *Tribolium*.
sundaicus, *Anopheles*.
Sunflower, *Hilda patruelis* on, in E. Africa, **233.**
Sweet Pea (see *Lathyrus odoratus*).
swynnertoni, *Glossina*.
Synphlebotomus, a new subgenus of *Phlebotomus*, **97.**
Syntomosphyrum, parasite of *Leucoptera* in E. Africa, **408.**
Systates articollis, on groundnuts in Tanganyika, **235.**

T.

tabaci, *Bemisia*.
tachinoides, *Glossina*.
taeniarostris, *Harpagomyia*.
Taeniorhynchus, in E. Africa, **454**; species of, in Sudan, **143.**
Tanganyika, Coccinellid predacious on red spider in, **307.**
Tasmania, Coccinellid predacious on mite in, **299.**
telarius, *Tetranychus*.
Teleopterus violaceus, parasite of *Leucoptera* in E. Africa, **406**; bionomics of, **413.**
Temperature, influence of, on *Musca domestica*, **339–357.**
Tenebroides mauritanicus, in stored groundnuts in E. Africa, **236.**
tenuirostris, *Ixodes* (see *I. trianguliceps*).
tessellatus, *Anopheles*.
testulalis, *Maruca*.
tetranychi, *Stethorus*.
Tetranychus bimaculatus (see *T. telarius*).
Tetranychus exsiccator, Coccinellid predacious on, in Hawaii, **299.**
Tetranychus pacificus, Coccinellid predacious on, on apple in U.S.A., **299.**
Tetranychus telarius (and var. *russeolus*), food-plants, distribution and predators of, **299.**
Tetranychus turkestani, Coccinellid predacious on, on cotton in Uzbekistan, **299.**
Tetranychus yothersi, Coccinellid predacious on, on avocado in U.S.A., **299.**
Tetrastichodes leucopterae, parasite of *Leucoptera* in E. Africa, **410**; bionomics of, **413.**
Tetrastichus, parasite of *Schematiza cordiae* in Trinidad, **218–219.**
thalassius, *Culex*.
Theileria parva, and ticks, **426.**
Thrips, Coccinellid predacious on, on *Sambucus* in Europe, **299.**

thusa, Tribolium.
tigripes, Culex (*Lutzia*).
Tilapia, predacious on mosquitos in Sudan, **142**.
Tobacco, development of *Ephestia elutella* on, **120–124**.
toroensis, Culex.
Tortrix dinota, on groundnuts in Uganda, **233**.
townsvillensis, Pericoma.
Traps, for *Glossina* in W. Africa, **491–528**.
trianguliceps, Ixodes.
Tribolium, synopsis and evolution of species-groups of, **13–55**; key to species of, **26**.
Tribolium alcine, sp. n., from Madagascar, **46**; characters of group of, **18**.
Tribolium anaphae, sp. n., from N. Nigeria, etc., **34**.
Tribolium antennatum, sp. n., from Australia, **54**.
Tribolium brevicornis, characters of group of, **15**.
Tribolium carinatum, sp. n., from Argentina, **30**.
Tribolium carinatum subsp. *dubium*, n., from Argentina, **32**.
Tribolium castaneum, in stored groundnuts in E. Africa, **236**; characters of group of, **19**.
Tribolium ceto, sp. n., from Madagascar, **48**.
Tribolium confusum, characters and bionomics of group of, **16–17**.
Tribolium cylindricum, sp. n., from Malaya, **52**; characters of group of, **20**.
Tribolium destructor, distribution of, **16**.
Tribolium dolon, sp. n., from Madagascar, **47**.
Tribolium downesi, sp. n., from Fr. Sudan, **40**; characters of group of, **17**.
Tribolium freemani, sp. n., from Kashmir, **50**.
Tribolium gebieni, **15**.
Tribolium giganteum, sp. n., from Kenya, **37**; characters of group of, **17**.
Tribolium indicum, in Fr. Somaliland, **44**; key to forms of, **44**.
Tribolium indicum f. *ares*, n., from Sudan, **44**.
Tribolium indicum f. *seres*, n., from Senegal, **44**.
Tribolium linsleyi, sp. n., from Mexico, **32**.
Tribolium madens, distribution of, **19**.
Tribolium myrmecophilum, in ants' nests, **21**; characters of group of, **21**.
Tribolium parallelus, in stored cereals, **16**.
Tribolium parki, sp. n., from Larat I., **51**.
Tribolium politum, sp. n., from Riouw Archipelago, **52**; characters of group of, **20**.
Tribolium semele, sp. n., from Fr. Sudan, **41**.
Tribolium sulmo, sp. n., from Abyssinia, **42**.
Tribolium thusa, sp. n., from S. Africa, **44**.
Tribolium waterhousei, sp. n., from Australia, **50**; characters of group of, **20**.
trifilatus, Culex.
Trinidad, proposed introduction of beetles from, into Mauritius against *Cordia*, **213, 217, 439**.
Trombicula, methods of trapping, **281–295**.
Trombicula deliensis, notes on rearing, **292–295**.
truncatus, Stethorus.
Trypanosomiasis, of cattle in E. Africa, **433**; and *Glossina* in W. Africa, **529**.
Tsetse-flies (see *Glossina*).
turkestani, Tetranychus.

U.

Uganda, mosquitos breeding in plant axils in, **185–212**; experiments with "Gammexane" and DDT against ticks in, **269–276, 277–279**.
unicavatus, Ixodes.
uniformis, Ficalbia (*Ingramia*); *Taeniorhynchus* (*Mansonioides*).
unilineatus, Aëdes (*Stegomyia*).
Union of Soviet Socialist Republics, Coccinellids predacious on mites in, **299**; new Coccids in, on imported bananas, **419–420**.
unipunctata, Estigmene.
univittatus, Culex.
Uranotaenia balfouri, in Sudan, **143, 146**.
Uranotaenia mashonaensis, in Sudan, **143, 146**.
Uranotaenia ornata var. *musarum*, breeding in plant axils in Uganda, **195–210**.
uriae, Ixodes.
utilis, Stethorus.

V.

vagans, Stethorus.
vagus, Anopheles.

variegatum, Amblyomma.
variegatus, Aëdes ; *Atoposoma.*
varuna, Anopheles.
vespertilionis, Ixodes.
Vicia spp. (Vetch), *Laspeyresia nigricana* on, in Britain, **67.**
Vigna unguiculata (see Cowpea).
Vines, Grape, Coccinellid predacious on mite on, in Europe, **299.**
vinsoni, Stethorus.
violaceus, Teleopterus.
vittatus, Aëdes (*Stegomyia*).
vivida, Parasa.

W.

Wheat, development of *Ephestia elutella* larvae on, **117–120.**
Winberry, relation of, to tick ecology in Wales, **331–337.**

X.

Xanthomonas malvacearum, infesting cotton, **554.**
Xanthosoma sagittifolium, mosquitos breeding in, in Uganda, **189–195.**

Y.

Yellow Fever, in Kenya, **489.**

Z.

Zophosis congesta, on groundnuts in Tanganyika, **234.**

INDEX TO NAMES OF PERSONS.

Afzal Husain, M., **304, 310.**
Alluaud, C., **39.**
Andrewes, H. L., **534, 535.**
Andrews, F. W., **155.**
Arthur, D. R., **321.**
Arthur, J. H., **266.**
Ashby, D. G., **83.**
Audy, J. R., **295.**

Bagster Wilson, D., **465.**
Basden, E. B., **117.**
Bathie, P. de la, **49.**
Beament, J. W. L., **359, 467.**
Bell, T. R., **534.**
Blewett, M., **124, 130.**
Bloss, J. F. E., **529.**
Blow, N. B., **3.**
Booth, C. E., **571.**
Borkhsenius, N. S., **417.**
Bose, M., **534.**
Bowen, E. G., **337.**
Bowring, **314.**
Britton, E. B., **311.**
Broderick, G. L., **241, 266.**
Busvine, J. R., **179.**
Buxton, P. A., **266, 295, 356, 383, 487.**

Callan, E. McC., **213.**
Cameron, M., **307.**
Champion, H. G., **313, 534, 535.**
Chelladorai, **537.**
Cherian, M. C., **310.**
Christophers, Sir R., **397.**
Cloudsley-Thompson, J. L., **386.**
Cockings, K. L., **281.**
Conway, W. M., **50.**
Cowley, L. F., **337.**

Dakshinamurty, S., **339.**
Dammerman, **54.**
Das, B., **534.**
Das, G. M., **312.**
Dawes, B., **337.**
d'Eca, L. Salazar, **571.**
Decorse, J., **46.**
de la Bathie, P., **49.**
de Maine, A. N., **571.**
Descarpentries, J., **47, 48.**
de Zeltner, F., **41.**
Diver, C., **266.**
Dodd, F. P., **55.**
Doherty, **534, 535.**
Dorea, J. B., **266.**
Douliot, **47.**
Downes, J. A., **41.**
Dunn, J. A., **83.**
du Toit, R., **504.**

Emden, F. van, **529.**
Evans, H. S., **35.**
Evans, J. W., **131.**

Fairman, H. D., **155.**
Fielding, W. L., **571.**
Fitzgerald, D. V., **307, 308.**
Fletcher, T. B., **534.**
Fox, D. G. R., **241.**
Fraenkel, G., **124, 130.**
Freeman, J., **50.**
Fryer, Sir J., **2.**
Fulani, Braimah, **527.**

Galley, R. A. E., **2.**
Garner, H. M., **2.**
Garnham, P. C. C., **489.**
Geay, F., **47.**
Geering, Q. A., **57.**
Gerber, J. H., **241.**
Gibbins, E. G., **186, 212.**
Gillett, J. D., **186, 212.**
Glauert, M., **2.**
Gordon, W. S., **295.**
Greenwood, W., **319.**

Haddow, A. J., **185.**
Hadjinikolau, J., **179.**
Hafez, M., **385.**
Hall, W. J., **297.**
Hargreaves, E., **305.**
Harper, J. O., **212.**
Harris, W. V., **232.**
Hart, G. F. W., **221.**
Hayward, K. J., **32.**
Heydon, G. A. M., **167, 176.**
Hinton, H. E., **13.**
Hirsch, G. P., **83.**
Hobby, B. M., **337.**
Hocking, K. S., **453.**
Höge, **34.**
Hopkins, G. H. E., **212.**

Husain, M. Afzal, **304, 310.**
Hutson, J. C., **536.**

Jackson, C. H. N., **441.**
Jackson, H. G., **130.**
Jarvie, **241, 243.**
Jepson, W. F., **231.**
Johnson, C. G., **356.**
Johnson, M. L., **397.**

Kapur, A. P., **297, 304, 310, 531, 534.**
Keisser, **35.**
Kennedy, J. S., **397.**
King, H. E., **539, 571.**
Kirkpatrick, T. W., **399, 416.**
Kluge, E. B., **405.**
Koebele, **316.**

Laws, S. G., **277, 434.**
Leeson, H. S., **221.**
Lefroy, H. M., **310.**
Lepesme, P., **14.**
Lewis, D. J., **133, 529.**
Linsley, E. G., **14.**
Liptrot, R. N., **2.**

Macan, T. T., **237.**
MacDonald, D., **571.**
MacInnes, D. G., **453.**
Mackerras, I. M., **177.**
Mackinnon, D. L., **337.**
McLennan, D. A., **266.**
MacLeod, J., **337.**
Madge, D., **309.**
Maine, A. N. de, **571.**
Mann, C. J., **51.**
Marsh, **2, 3.**
Marshall, Sir G. A. K., **35, 46.**
Massee, A. M., **487.**
Mavromoustakis, G. A., **304.**
Mellanby, K., **295.**
Mer, G. G., **266.**
Miller, D., **318.**
Misra, C. S., **310.**
Morris, K. R. S., **491.**
Morris, M. G., **491.**
Mortimer, D. A., **254, 266.**
Mossi, Bukari, **527.**
Muggeridge, J. A., **318.**
Muir, F., **52.**
Munro, J. W., **130.**
Murtaza, A., **310.**

Nixon, G. E. J., **83.**
Noble, A. H., **231, 232, 234.**
Notley, F. B., **232, 399.**

Odobill, J., **527.**
Oliveira Santos, F., **571.**
O'Sullivan, P. J., **159.**
Oxley, T. A., **120, 130.**

Park, T., **52.**
Parker, A. H., **387.**
Parnell, F. R., **539, 571.**
Pendlebury, H. M., **53.**
Petit, **47.**
Pettman, **240.**
Potts, W. H., **449.**
Pullin, C. G., **2.**

Raffray, **43.**
Rainsford, R. W. S., **266.**
Ramsay, C. G., **256.**
Rao, R. Y., **310.**
Rao, V. P., **534, 536, 537.**
Richards, H., **155.**
Richards, O. W., **120, 130, 531.**
Richards, W. S., **286, 295.**
Riley, N. D., **297.**
Ripper, W. E., **1.**
Roberts, **241.**
Roberts, F. H. S., **159.**
Roberts, H., **534.**
Roepstorff, **536.**
Rogers, G., **534.**
Ross, E. S., **14, 34.**
Russell, Sir A., **356.**
Ruston, D. F., **539.**

Said Mohammed, **416.**
Salazar d'Eca, L., **571.**
Samuel, C. K., **310.**
Santos, F. Oliveira, **571.**
Saunders, C. J., **53.**
Scott, H., **311.**
Senior White, R., **264, 266.**
Senkubuge, Om. Y., **212.**
Shanks, R., **241.**
Shepherd, **241, 245, 254.**
Simmonds, F. J., **213** (note), **217, 435.**
Simmonds, H. W., **319.**
Singh, **243.**
Singh, D. P., **310.**
Smith, A., **14.**
Smith, R. F., **14.**
Soper, W. J., **83.**
Squires, **2.**
Stebbing, E. P., **535.**
Symes, C. B., **208.**

Taylor, T. H. C., **37, 297, 531.**
Terrell, C. G., **255.**
Theilman, **3.**
Theodor, O., **85, 221.**

Thomas, A. S., **212.**
Thomas, H. M., **286, 295.**
Thompson, J. L. Cloudsley-, **386.**
Toit, R. du, **504.**
Topping, **241.**
Trewavas, E. M., **155.**
Tucker, R. W. E., **307.**
Tudor, P., **1.**
Turner, R. E., **46.**

Upadhyayula, V. C., **536.**

van Emden, F., **529.**
Veitch, R., **319.**
Vinson, J., **309.**

Wagner, E. R., **32.**
Wakefield, C. M., **318.**
Walker, **314.**
Wallace, A. R., **53.**
Waloff, N., **117.**
Ward, T. P., **221.**
Waterhouse, D., **51.**
Waterhouse, J. A. H., **397.**
Watson, G. I., **238, 241, 255.**
White, R. Senior, **264, 266.**
Wigglesworth, V. B., **383, 487.**
Willis, C. D., **316.**
Wilson, D. Bagster, **465.**
Wilson, S. G., **269, 277, 423.**
Worth, A. H., **3.**
Wright, D. W., **57.**

Yates, F., **448.**

Zeltner, F. le, **41.**

4°
1p6